GENETICS

A periodical record of investigations bearing on heredity and variation

Founded in 1916 by GEORGE H. SHULL

with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

VOLUME 46 MAY, 1961 NUMBER 5

TABLE OF CONTENTS

WHITTINGHILL, MAURICE, and BONNY MORGAN LEWIS, Clustered crossovers from male Drosophila raised on formaldehyde media . . 459

HESSLER, ANITA Y., A study of parental modification of variegated position effects 463

TUVESON, R. W., and E. D. GARBER, Genetics of phytopathogenic fungi. IV. Experimentally induced alterations in nuclear ratios of heterocaryons of *Fusarium oxysporum* f. *pisi* 485

CHOVNICK, ARTHUR, The garnet locus in *Drosophila melanogaster*. I. Pseudoallelism 493

RUSSELL, LIANE BRAUCH, and JEAN W. BANGHAM, Variegated-type position effects in the mouse 509

KOJIMA, KEN-ICHI, and THERESE M. KELLEHER, Changes of mean fitness in random mating populations when epistasis and linkage are present 527

STORMONT, CLYDE, W. J. MILLER and YOSHIKO SUZUKI, The S System of bovine blood groups 541

CARSON, HAMPTON L., Relative fitness of genetically open and closed experimental populations of *Drosophila robusta* 553

SAND, SEAWARD A., Effects of flower node position on the mutable *V* and stable *R* loci in a clone of Nicotiana 569

MACINTYRE, PAMELA A., Spontaneous sex reversals of genotypic males in the platyfish *(Xiphophorus maculatus)* 575

PUBLISHED MONTHLY AT AUSTIN, TEXAS
BY GENETICS, INC.
(Date of issue, June 1, 1961)

EDITORIAL BOARD

CLARENCE P. OLIVER, *Managing Editor,*
WILSON S. STONE, *Managing Editor,*
University of Texas

GEORGE W. BEADLE
California Institute of Technology

R. ALEXANDER BRINK
University of Wisconsin

WILLIAM E. CASTLE
University of California

JAMES F. CROW
University of Wisconsin

EVERETT R. DEMPSTER
University of California

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

A. D. HERSHEY
Carnegie Institution of Washington

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
Indiana University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

CURT STERN
University of California

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Wisconsin

VOLUME 46 MAY, 1961 NUMBER 5

GENETICS is a monthly journal issued in annual volumes of about 1000 pages each.

Subscription, $12.00 net a year for complete volumes (January–December). Foreign postage, $1.00 additional. Single copies, $1.50 each, postpaid. GENETICS is sold by volume only. All subscriptions must begin with the January issue.

As available, back volumes 1 through 44 may be had at $14.00 each and Volume 45 may be purchased for $28.00. Single issues of previous volumes are $2.50 per copy, postpaid. The Business Office will supply information on request as to volumes and numbers available.

Business Correspondence, including change of address, etc., should be addressed to GENETICS, INC., Business Office, Experimental Science Bldg. 122, University of Texas, Austin 12, Texas.

Remittances should be made payable to **GENETICS, INC.**

Correspondence concerning editorial matters should be addressed to the EDITORS OF GENETICS, Experimental Science Building 122, University of Texas, Austin 12, Texas. 3579 should be sent to this address also.

Second-class mail privileges authorized at Austin, Texas.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

CLUSTERED CROSSOVERS FROM MALE DROSOPHILA RAISED ON FORMALDEHYDE MEDIA

MAURICE WHITTINGHILL AND BONNY MORGAN LEWIS[1]

Department of Zoology, University of North Carolina, Chapel Hill, N. C.

Received January 16, 1961

THE relation of mutagenesis to crossover induction is of special interest in the study of chromosomes. Crossover inducers among the physical mutagens include temperature extremes, X-rays, gamma rays and UV. Crossover induction has also been reported for several chemical mutagens: $MnCl_2$ and ethylenediaminetetraacetic acid (EVERSOLE and TATUM 1956), dihydroxydimethyl peroxide (SOBELS and VAN STEENIS 1957), formaldehyde injected into adult males (SOBELS 1956), and nitrogen mustard, methyl-bis(b-chloroethyl) amine (WHITTINGHILL 1948). It would be desirable to find a crossover inducer which is not mutagenic, but for the present our interest centers upon those substances and environmental conditions which have a dual effect, crossover induction and mutation. A preliminary report has been made by LEWIS (1957).

This study was undertaken (1) because the demonstration by AUERBACH (1956) of mutagenesis after formaldehyde treatment of larval Drosophila suggested that crossover induction might also be possible and (2) because any crossing over might occur early in gonial cells as in the experiments with a physical mutagen, gamma rays (WHITTINGHILL 1951) or might be delayed until the usual time for crossing over, at meiosis.

MATERIALS AND METHODS

A crossover-selector system which screens for exchanges in the third chromosome pair of *Drosophila melanogaster* was used (WHITTINGHILL 1950). Heterozygous males come from an incompletely balanced *My Gl* + +/+ + *Sb Ubx* stock in which homozygous marker flies die. The dominant genes *My* (Minute(3)y) and *Gl* (Glued) are closely linked in coupling phase, and in the other third chromosome *Sb* (Stubble) and *Ubx* (Ultrabithorax) are also closely coupled at 17 crossover units away from the *Gl* locus. The sensitive spindle attachment region lies between *Gl* and *Sb*. Such *My Gl* + +/+ + *Sb Ubx* males mated to *My* + *Sb* +/+ *Gl* + *Ubx* females (from an easily maintained "alternated" stock) have all of their noncrossover offspring killed by homozygosity for one or another of the four dominant markers. The many eggs which are crossovers in the long *Gl–Sb* interval also become zygotic lethals, and crossover eggs of rarer types give rise to adults among less than one percent of all eggs fertilized.

[1] Present address: Mrs. K. B. Lewis, Department of Human Genetics, Johns Hopkins Hospital, Baltimore 5, Md.

Yet 50 percent of crossover sperm will be + + + + and will give rise to adult offspring showing two markers from the mother, rarely one or three. Further details of this screening method have been published in WHITTINGHILL 1951, page 349. The genotypes of recovered adults may be confirmed by progeny testing. The presence of larvae attests to the fertility of a mating, because homozygous lethals such as *My*/*My* remain mobile for a week or more as first instars before they die. Hence large numbers of culture vials may be followed in a single experiment.

The culture medium and treatment methods of AUERBACH and MOSER (1953) were used. Concentrations of formaldehyde in the medium were 0.20 percent and 0.25 percent. First instar or second instar larvae, *My Gl*/*Sb Ubx*, were transferred to fresh control or formaldehyde medium at the proper age. The original culture plates received live yeast but the transfer bottles with or without formalin received none.

On the day of their emergence, males from the treated and from the control cultures were coded and then mated individually to three virgin "alternated" females per vial for three days (Brood 1). Then the females were subcultured to add to Brood 1 data, and the males were remated to three more virgin females for six days (Brood 2).

RESULTS AND DISCUSSION

Among the controls one of 111 fertile (larvae-producing) males had a single Glued Stubble/++ offspring, evidently from a crossover + + + + sperm and a *Gl Sb* egg. It was possible to estimate the numbers of sperm tested by classifying 261 other adults, which constituted all the adult progeny from over 200 cultures with live larvae. All came from the rarer types of crossover eggs, mostly *Sb* or *Gl* single crossovers and occasionally *Ubx* or *M(3)y* double crossovers, and their genotypes were generously sampled by progeny testing. If their total occurrence is taken from standard map distances to be 0.45 percent, then an estimated 58,000 zygotes started development. The one crossover sperm indicated, with its unrecoverable complementary *My Gl Sb Ubx* chromosome, a frequency of .00003 spontaneous recombination. PATTERSON and SUCHE (1934) have reported one spontaneous crossover, suitably flanked by several recessive markers, among 8329 control flies. P. T. IVES (personal correspondence) also has encountered spontaneous crossovers from males.

Among 150 tested males raised on formaldehyde food medium 15 flies had one or more crossover sperm as evidenced by the breeding test. The highest frequency of affected males was among those transferred as second instars to the 0.25 percent formaldehyde food, Series E & K, Table 1. In these two series crossover offspring were recovered in both the early and the later broods, a fact which indicated either a long delay of effect upon meiosis or an early effect upon gonia followed by rapid or slow progress toward and through gametogenesis.

The recovery of several crossovers, constituting a "cluster", from the same treated male is shown in Table 1 wherever there are parentheses. The sizes

TABLE 1

Crossover-producing males following transfer of M(3)y Gl+ +/+ + Sb Ubx *Drosophila larvae to medium containing low or zero concentrations of formaldehyde*

Series	Larval instar transferred	HCOH concentration Percent	No. males mated	No. males fertile	No. of males with crossovers in Brood 1: 1–3 days	Brood 2: 4–9 days	Summary: Males with crossovers in any brood No.	Percent
H,J,F,L	1st,2nd	0	117	111	1	0	1	0.9
G	1st	0.20	27	23	0	0	0	0
I	1st	0.25	27	26	2(1,4)*	0	2	7.7
A	2nd	0.20	41	35	0	2(3,4)	2	5.7
E	2nd	0.25	42	29	1	3(1,1,2)	.	. .
K	2nd	0.25	45	37	4(1,1,2,2)	3(1,9,19)	11	16.7
Treated Series Totals			182	150	7	8	15	10.0

* Parentheses enclose the number of similar crossover offspring (a "cluster") in single families. No cluster was distributed over both broods in these experiments.

of the clusters were 19 in one culture, nine in another, four, three, and the minimum of two in several families. Such multiple recovery is remarkable because most of the treated males, 135 out of 150 bred, had no crossovers, and yet those 15 which did have any crossovers had a crossover cluster in eight of the 15 sets of progenies. This distribution is decidedly non-Poisson, and it has been attributed to variable gonial multiplication of the products of occasional crossing over after such exchanges had occurred as few as 15 times among the 150 tested fathers. Contamination as a source of the observed bunches of crossovers may be ruled out, first because early contamination by wild males would be expected to produce even larger batches of similar offspring, and secondly, because there was no source for later invasions of small numbers of the right phenotypes to be confused with the zygotes expected from the crossover-selector matings. Furthermore the experiments were performed in five different months with intervals between experiments, yet small or medium sized clusters occurred in all of the five treatment series except Series G.

The customary expression of the result in terms of crossover frequency among gametes may be made, but such will be less accurate than for meiotic crossovers chiefly because of the easy variation in the size of a cluster of crossover sperm but also because of the intensive screening. As to the latter point, the 1.8 percent crossover eggs expected from the lengths of the *My–Gl* plus the *Sb–Ubx* regions must first be cut in half for the unrecoverable chromosomes with three markers sure to be homozygous lethal with any sperm and again cut in half to discount lost homozygotes from *Gl* egg/*My Gl* sperm and *Sb* egg/*Sb Ubx* sperm. Therefore 153 classified adults indicate 306 single marker chromosomes which in turn represent 612 estimated complementary crossovers in either of the small flanking regions. The 612 crossover eggs would be 1.8 percent of 34,000 zygotes. Meanwhile 104 crossover sperm (estimated from 52 classified adults) were produced non randomly within and among families. These crude calculations lead to an

induced crossover sperm frequency estimate of 0.3 percent but this figure is not linearly related to the frequency of the exchange process in the cells of the male larvae. However, the estimate is one or two orders of magnitude above the spontaneous crossover sperm frequency indicated among our control vials.

SUMMARY

The relation of crossing over to meiosis and to gonial mitosis in males was studied by the chemical treatment of young *Drosophila melanogaster*. First instar or second instar larvae transferred onto culture medium containing formaldehyde later produced crossover sperm in 10 percent of the 150 males bred. Both first and second instar larvae placed on 0.25 percent formaldehyde medium later produced crossovers, and second instars placed on 0.20 percent HCOH also produced crossover sperm. The crossover offspring were recovered in both of two broods. They appeared in clusters as often as singly within a family and brood. The clusters of similar crossovers were both numerous enough and large enough to attribute each cluster to a single exchange event in one gonial cell of each affected male without recourse to any explanation involving meiotic events. The screening procedure for crossovers required that any computation of crossover frequency be an indirect one; the induced crossovers were estimated to comprise 0.3 percent of sperm. One crossover sperm was detected from one of 111 control males.

LITERATURE CITED

AUERBACH, C., 1956 Analysis of the mutagenic action of formaldehyde food. III. Conditions influencing the effectiveness of the treatment. Z. Ind. Abst. Vererb. **87**: 627–647.

AUERBACH, C., and H. MOSER, 1953 An analysis of the mutagenic action of formaldehyde-food. I. Sensitivity of Drosophila germ cells. Z. Ind. Abst. Vererb. **85**: 479–504.

EVERSOLE, R. A., and E. L. TATUM, 1956 Chemical alteration of crossing over frequency in Chlamydomonas. Proc. Natl. Acad. Sci. U.S. **42**: 68–72.

LEWIS, BONNY MORGAN, 1957 Formaldehyde-induced crossing over in *D. melanogaster* males. Drosophila Inform. Serv. **31**: 130.

PATTERSON, J. T., and META L. SUCHE, 1934 Crossing over induced by X-rays in Drosophila males. Genetics **19**: 223–236.

SOBELS, F. H., 1956 Studies on the mutagenic action of formaldehyde in Drosophila. II. The production of mutations in females and the induction of crossing over. Z. Ind. Abst. Vererb. **87**: 743–752.

SOBELS, F. H., and H. VAN STEENIS, 1957 Chemical induction of crossing over in Drosophila males. Nature **179**: 29.

WHITTINGHILL, MAURICE, 1948 The effects of methyl-bis (b-chloroethyl) amine upon recombination. Genetics **33**: 634.

1950 Two crossover-selector systems: new tools in genetics. Science **111**: 377–378.

1951 Some effects of gamma rays on recombination and on crossing over in *Drosophila melanogaster*. Genetics **36**: 332–355.

A STUDY OF PARENTAL MODIFICATION OF VARIEGATED POSITION EFFECTS[1, 2]

ANITA Y. HESSLER[3]

Department of Zoology, University of Chicago, Chicago 37, Illinois

Received February 19, 1960

THE study of genetic modification of the interruptions in homogeneous development of pigment which characterize white-variegated position effects in *Drosophila melanogaster* produces a variety of information. The compound eyes, testis sheath, and malpighian tubules in the mutant white (w, 1–1.5) are colorless, whereas the eyes are red and the sheath and tubules uniformly yellow in wild-type flies. White variegated flies possess at least one w^+ allele which, through a chromosomal rearrangement R(w^+), is associated with heterochromatin (see review on position effect by LEWIS 1950) and accompanies the development of the variegated pigmentation. A R(w^+)/w fly will develop eyes wherein only part of the pigment cells form the products associated with visible pigment; some cells do not, and appear colorless. The other two tissues have patches of yellow or colorless cells (DEMEREC and SLYZINSKA 1937). The w allele completely blocks production of both brown pigment components (ommochromes) and red pigment components (drosopterins) of the wild-type eye. In the testis sheath of wild-type *D. melanogaster* certain pteridines develop, whereas the ommochromes and drosopterins do not. As the yellow pigment of the testis sheath is thought to be mainly sepia pteridine (GRAF and HADORN 1959) which is regarded as a substance produced in the synthetic chain leading to formation of drosopterins in eyes (FORREST and MITCHELL 1955), a comparison of the pteridines in these two tissues in white-variegated flies with their characteristically variable pigmentation might indicate the extent of correlation of pigment development in eyes and testis sheath.

The cause of the cell-to-cell somatic variation is unknown, although certain environmental and genetic factors can modify the variegation toward either a preponderance of unpigmented tissue or of pigmented tissue. For example, by adding heterochromatin to the genome in the form of fragments or whole Y chromosomes, genetic modification is realized which usually results in more pigmentation (GOWEN and GAY 1933; SCHULTZ 1936; BAKER and SPOFFORD 1959). This phenomenon was utilized here in analyzing the development of eye and testis sheath pigment simultaneously in males with one or two Y chromosomes.

[1] Accomplished on Contract No. AT(11-1)-431 for the Atomic Energy Commission.

[2] Submitted in partial fulfillment of requirements for the degree of Doctor of Philosophy in Zoology at the University of Chicago.

[3] Present address: Marine Biological Laboratory, Woods Hole, Massachusetts.

BAKER and SPOFFORD (1959) and SPOFFORD (1959) in their studies of white variegation found unusual parental effects on the amount of pigmentation in eyes of variegated offspring. The studies being reported in particular amplify, quantitate, and extend the unusual findings.

MATERIALS AND METHODS

Stocks: The w^+ allele employed in this study is the same one used by BAKER and SPOFFORD (1959), Dp(w^m)264–58a. This duplication is a 20-band segment of the X chromosome containing w^+ (close to one end) which has been relocated in inverted order within the proximal heterochromatin of 3L (SUTTON 1940). Although originally carried in a free X stock by DR. EILEEN S. GERSH, the duplication was introduced by DRS. BAKER and SPOFFORD in 1956 into two lines which have been kept separate since that time—one had free X chromosomes, the other attached-XY, attached-X chromosomes. The duplication in the former line is denoted by Dp^f; in the latter line, by Dp^a. The distinction implied by these symbols represents certain highly significant differences in behavior or state between Dp^f and Dp^a which were discovered during the course of this investigation.

In the experiments detailed below the Dp^a and Dp^f were contributed initially by one $\underline{yw}$/Y; Dp^a/Dp^a female and one yw/yw; Dp^f/Dp^f female respectively. The inbred white stock which were employed for testcrosses were (1) attached-X, attached-XY stock; $\underline{yw}$/Y females, $\widehat{XY}w$/Y males, and (2) a free-X yw stock (y = yellow body, 1–0.0; w = white eye, 1–1.5; $\underline{yw}$ = attached-X; $\widehat{XY}w$ = $Y^sX{\cdot}Y^L$.*In(1)EN* = $Y^s w\, y{\cdot}Y^L y^+$, see LINDSLEY and NOVITSKI 1959).

Flies were reared at 23±1°C on a culture medium modified slightly from that described by CARPENTER (1950). Pair matings of genotypes being compared were made up simultaneously on medium from the same batch. One or two replicates of each set of matings were made, each replication in sufficient numbers to yield at least ten fertile sets of parents. Two sets of smaller size, five and seven pairs, were obligatory in two cases because of infertility. The males used for chromatography were obtained by sampling the progeny of each pair in each replication at least once in the course of making daily collections. Sampling was random in the sense that the flies collected were simply those which eclosed in a given two-hour period after clearing of culture vials. The number of progeny produced by each pair mating was 50–60 regardless of the combination of parents used except MP-13 as noted later.

Chromatographic techniques: The eye pteridines which were measured are the drosopterins (DP), sepia pteridine (SP), and 2-amino-4-hydroxypteridine plus biopterin (HB_1 and HB_2). In the testis sheath SP, $HB_1 + HB_2$, and *iso*xanthopterin (IX) were measured (abbreviations as in ZIEGLER-GÜNDER and HADORN 1958). The drosopterins refer to a complex of at least three compounds which comprise the red pigment (VISCONTINI, LOESER and KARRER 1958). Sepia pteridine is probably responsible for yellow pigment of the testis sheath; at least it is the major constituent (GRAF and HADORN 1959). In my experiments HB_1 and

HB_2 were inseparable on one-way chromatograms. Their identity and relative abundance in eyes or testis were determined by running two-way descending chromatograms of samples of either ten heads or 12 testes (first solvent: propanol-7 percent NH_4OH, 2:1; second solvent: butanol-glacial acetic acid-water, 4:1:5). Comparisons were then made between the location of known 2-amino-4-hydroxypteridine added as a pure substance and the location of biopterin as accumulated in the eyes of the mutant sepia. It was found that the major part of the HB fluorescence in eyes and testis sheath was contributed by HB_2—biopterin. In variegated eyes HB_2 was distinctly in execess of the normal wild type amount (BAKER and SPOFFORD 1959), whereas 2-amino-4-hydroxypteridine was somewhat reduced. Sepia pteridine is present in excessive amounts, and drosopterin and *iso*xanthopterin are present in general in lower amounts in variegated eyes as compared with wild type. (For studies on the pteridines of Drosophila, see HADORN and MITCHELL 1951; FORREST and MITCHELL 1954a, 1954b, 1955; FORREST, GLASSMAN and MITCHELL 1956; FORREST, HATFIELD and VAN BAALEN 1959; VISCONTINI, LOESER and EGELHAAF 1956; VISCONTINI, LOESER and KARRER 1958; VISCONTINI and RASCHIG 1958; VISCONTINI 1958. For more general papers see Ciba Foundation Symposium on Chemistry and Biology of Pteridines, 1954; ALBERT 1954; ZIEGLER-GÜNDER 1956; HADORN 1959).

The males chromatographed were aged four to five days, dissected in Drosophila saline solution, and the samples—heads and testes—placed on Whatman No. 1 filter paper (46 cm × 57 cm) prepared for descending chromatography. Fifteen to 20 heads were crushed individually 1 cm apart, and the testes corresponding to the heads were placed in the same order on the same sheet of paper. Paper blanks were also run on each sheet. The solvent was normal propanol:7% NH_4OH (2:1). The paper was equilibrated with this solvent mixture for two to three hours prior to the chromatographic run of 16 hours in the dark which achieved adequate separation of the fluorescent components. The paper was air-dried in a laboratory hood and observed under UV light emitted by a Shannon lamp. The appropriate spots were marked, cut out, and eluted separately in 1 ml of dilute ammonia (1%) in individual stoppered vials for two hours; then the papers were removed.

Fluorescent measurements were made with a Farrand (Type A) photofluorometer using as an arbitrary fluorescent standard 1 μgm of anthranilic acid per 1 ml elution solvent. The galvanometer was adjusted to give a reading of two (on the 1–10 scale) for the 1 ml of standard before each set of readings. Thus all measurements are in terms of fluorescent units of this arbitrarily chosen standard. A primary filter, Corning No. 9863, was inserted between the UV source and sample, while secondary filters were placed between phototube and sample. The combination of No. 3385 and No. 3389 secondary filters was used to measure drosopterin and sepia pteridine, and the combination of No. 3385 and No. 4308 was used to measure *iso*xanthopterin and the HB complex. All readings were corrected by subtraction of the fluorescent value of a paper blank which had been cut from the chromatography paper at the same level as the sample.

RESULTS

Mating plan: A detailed diagram of the mating scheme is presented in Figure 1. In order to distinguish the immediate parental source of the Dp(w^m), the convention was employed of placing a maternally-inherited duplication to the left of the diagonal and a paternally-inherited duplication to the right, e.g. $\underline{yw}$/Y; Dp^a/+ or $\underline{yw}$/Y; +/Dp^a.

The expression of white-variegated position effects can be modified in many ways. The mating plan outlined in Figure 1 was designed to provide an organized framework for observing the operation of three particular modifying effects—the parental source effect, the effect of extra heterochromatin, and the effect of homozygous *vs.* heterozygous mothers. The plan was designed to produce information which might clarify the relationship of one modifying effect to another. In order to follow readily the various sets of comparisons being made, the chart has been labeled in this manner: the P and M series refer to patrilineal and matrilineal stocks where the Dp is maintained in either males or females, respectively, by crossing each generation to appropriate white stocks. The numerals designating series 1 and series 2 divide the M and P series of $\widehat{XY}w$/Y (series 1) from the M and P series employing yw/Y males (series 2), thus distinguishing the effect of extra heterochromatin. Finally, MP-1 and MP-2 identify lines established by certain deliberate combinations of the M and P lines to produce females homozygous for the duplication. The reasons for this plan will now be explained.

It is known that white-variegated progeny of the same genotype may have drastically different phenotypic expression depending upon whether the Dp was contributed by the mother or by the father. This effect of parental source of Dp is illustrated by the variegated progeny of $\underline{yw}$/Y; Dp^a/+ ♀♀ and $\widehat{XY}w$/Y; +/+ ♂♂, which develop far less pigment in their eyes than do the progeny of $\underline{yw}$/Y; +/+ ♀♀ × $\widehat{XY}w$/Y; +/Dp^a ♂♂ (Spofford 1959). In addition, daughters of these two crosses show significant differences in penetrance of Dp^a. Chromatographic assays of pteridines in eye and testis tissues of males from these reciprocal crosses were made. Are the quantitative differences exhibited in the eyes of the two kinds of sons paralleled by differences in testis sheath pteridines? It is of interest from a developmental standpoint to find whether both tissues are being similarly affected by the parental source of the duplication.

The mating plan as designed permits study simultaneously of a second effect —that of extra Y heterochromatin. As it was unknown whether the parental source effect of Dp obtains in crosses using attached-X females but free X males, the matings were arranged so as to provide separate matrilineal and patrilineal lines of the duplication in both $\widehat{XY}w$/Y males (series 1) and free X males (series 2). By examining the parental source crosses in terms also of additional heterochromatin, one might expect to resolve certain regularities in the effects on pigment formation. It was hoped that observations on penetrance and analysis by chromatography might establish the action of additional heterochromatin of the $\widehat{XY}w$ chromosome in enhancing pigment production in testes as well as in eyes.

FIGURE 1.—An outline of the mating plan employed in producing the various parental stocks and progeny. For details on nomenclature, see Materials and Methods. The mottled males used in matings MP-1 and MP-2 were siblings of the males used in P-1 and P-2, respectively. Parentheses enclose certain groups of males that were chromatographed.

The mating plan provides for testing of a third modifying effect—that of augmented pigment production in progeny (heterozygous for Dp) of a *yw*/Y; Dp/Dp mother compared with the progeny of a *yw*/Y; Dp/+ mother. Series MP-1 and MP-2 in Figure 1 denote the deliberate recombination of matrilineal and patrilineal stocks to produce homozygous Dp females whose progeny could be compared with those of heterozygous Dp females. As in the case of other modifiers, inspection of progeny for penetrance of the duplication is an important criterion; and employment of chromatography of males proved especially useful, since phenotypic differences otherwise difficult to characterize without a large number of progeny could be easily detected and more subtly analyzed.

In summary, the mating plan was to provide simultaneous samples of all the kinds of flies to be compared. However, the experimental results also disclosed what appear to be real and significant differences between the way Dp^a and Dp^f act. Thus, the plan did not accomplish all that was intended. Sufficient data were accumulated to check the parental source effect completely in the $\widehat{XY}w$/Y series, but not in the *yw*/Y series. Comparative effectiveness of extra heterochromatin could be examined in both $\widehat{XY}w$/Y and *yw*/Y matrilineal series (M-11 and M-21, respectively), but it could not be examined in corresponding patrilineal matings (P-11 and P-21), where the difference between Dp^a and Dp^f invalidates such comparison. The desired comparisons of effects of homozygous Dp mothers *versus* mothers heterozygous for Dp are valid only in the Dp^a series (MP-1). Although the data collected from the MP-2 series are not directly comparable, they provide strong evidence that the original duplication now exists in two states, Dp^a and Dp^f.

Modifying effects associated with Dp^a: The parental source effect of Dp^a may be best described in two ways: the difference in penetrance of Dp^a in daughters and the difference in degree of expression in sons as determined chromatographically. As shown in Table 1, the mottled daughters of P-11 (*yw*/Y; +/Dp^a) comprise a far larger percentage (47.7%) of the total female progeny (white plus mottled) than do *yw*/Y; Dp^a/+ daughters of M-11 (29.4%). In addition, more pigment developed in P-11 than in M-11 daughters. Mottled sons from both crosses occurred in numbers insignificantly different from the expected 50 percent, but, as in their female siblings, M-11 sons ($\widehat{XY}w$/Y; Dp^a/+) exhibit markedly reduced pigment development compared to the $\widehat{XY}w$/Y; +/Dp^a P-11 sons. (This will be elaborated on later in the paper.)

When the MP-1 matings were made, a number of variegated females were produced which may have received Dp^a from either a mother or a father. (Note also that this group differs from the M-11 and P-11 ones in that the egg or sperm bearing the wild-type third chromosome is contributed by a Dp^a-bearing parent rather than by a homozygous testcross stock of *yw*/Y; +/+, $\widehat{XY}w$/Y; +/+.) Since the mottled daughters (*yw*/Y; Dp^a/+ and *yw*/Y; +/Dp^a) of MP-1 showed a range of mottled phenotypes which could not be placed into distinct sets, it was impossible to designate the parental source of Dp^a. In addition, a number of Dp^a/Dp^a homozygous females were expected to survive, and their identification is inconclusive except by progeny test, although they tended to be somewhat

TABLE 1

Summary of genetic characteristics of Dp^a and Dp^f white-variegated progeny of mating plan with pteridine analysis of some males

Mating	Daughters: Genotype	Daughters: No.	Daughters: Percent w^{ss}	Sons: Genotype	Sons: Tissue*	Sons: No.	Sons: Percent w^{ss}	Pteridine analysis: No.	Drosopterin Mean	Drosopterin S.E.	Sepia Pteridine Mean	Sepia Pteridine S.E.	Biopterin + 2-amino-4-hydrox. Mean	Biopterin + 2-amino-4-hydrox. S.E.	*Isoxanthopterin* Mean	*Isoxanthopterin* S.E.
M-11	*yw*/Y;Dp^a/+	921	29.4	$\widehat{XY}w$/Y;Dp^a/+	E	880	48.7	57	0.247	±.002	1.121	±.106	1.757	±.155	.....	
					T	880	50.1	58	.....		0.814	±.076	1.010	±.085	4.286	±.480
MP-11, 12	*yw*/Y;Dp^a/+	1067	29.1	$\widehat{XY}w$/Y;Dp^a/+	E	1206	48.1									
P-11	*yw*/Y;+/Dp^a	815	47.7	$\widehat{XY}w$/Y;+/Dp^a	E	868	50.8	59	2.954	±.169	3.732	±.100	4.949	±.149	.....	
					T			59	.....		2.309	±.089	2.581	±.118	17.237	±.542
M-21	*yw*/Y;Dp^a/+	1018	25.3	*yw*/Y;Dp^a/+	E	959	0	12	0.000		trace		trace		.....	
					T	433	15.5	12	.....		0.350	±.357			0.367	±.171
MP-21	*yw*/Y;Dp^a/+	185	29.7	*yw*/Y;Dp^a/+	E	196	0									
					T	96	0									
MP-211	*yw*/Y;+/Dp^a	301	48.2	*yw*/Y;+/Dp^a	E	298	4.0									
P-21	*yw*/Y;+/Dp^f	1746	50.3	*yw*/Y;+/Dp^f	E	1629	23.2	17	0.153	±.030	0.557	±.137	1.041	±.247	.....	
					T	1629	48.0	51	.....		1.567	±.117	2.061	±.149	6.400	±.491
MP-22	*yw*/Y;Dp^f/+	286	59.8	*yw*/Y;Dp^f/+	E	295	45.1	15	0.285	±.052	0.600	±.178	1.600	±.452	.....	
					T	125	58.4	15	.....		.....		.....		7.857	±.504
MP-13 Dp^a/Dp^a mother	*yw*/Y;Dp^a/+	54	100.0	$\widehat{XY}w$/Y;Dp^a/+	E	53	100.0	21	0.771	±.154	1.605	±.206	2.829	±.367	.....	
					T			12	.....		2.450	±.310			7.133	±1.297
MP-23 Dp^a/Dp^f mother	*yw*/Y;Dp^a/+	168	100.0	*yw*/Y;Dp^a/+	E	242	65.8	23	0.496	±.138	0.600	±.143	1.481	±.375	.....	
	yw/Y;Dp^f/+			*yw*/Y;Dp^f/+	T	242	100.0	30	.....		2.980	±.271			5.963	±.664
MP-231 light mother	*yw*/Y;Dp^a/+	929	15.6	*yw*/Y;Dp^a/+	E	915	0									
MP-232 dark mother	*yw*/Y;Dp^f/+	377	54.4	*yw*/Y;Dp^f/+	E	398	42.5									

* E = eyes; T = testes.

more fully pigmented as a group than were the heterozygotes. As noted in Table 1, the daughters of the females which turned out to be heterozygous (MP-11, 12) resemble those of M-11 in that the penetrance of the duplication is essentially identical. Also, the expression of pigment in general among the progeny of MP-11, 12 was more similar to that of M-11 progeny than to P-11 progeny, with only a few exceptions showing much pigment. Thus, there is no evidence of a grandparental source effect.

Unlike similar tests on the M-11 and P-11 progeny, a chi-square test for heterogeneity within the 41 pairs comprising MP-11, 12 reveals very significant heterogeneity as to percentages of variegated flies from one mating to another, which could be largely accounted for by four exceptional pairs. Out of a total of 90 female progeny produced by these four pairs 55 were mottled, whereas 95 of the 99 sons were mottled. These numbers yield percentages (61% female, 96% male which are significantly different from the percentage of mottled progeny of the other 37 pairs (26.2% female, 43.8% male). The progeny of the exceptional pairs also developed more pigment. It is not known if these four exceptional pairs represent matings of mothers homozygous for Dp^a(MP-13) wherein a portion of the daughters and four of the sons are actually heterozygous for Dp^a but unpigmented or whether the exceptional progeny are actually from heterozygous mothers and the white-eyed progeny are not being recovered as adults in the usual numbers. Excluding these four exceptions, it may be said that most of the heterozygous daughters of the MP-1 mating seem to produce progeny which are indistinguishable from matrilineal (M-11) crosses and thus show no grandparental source effect attributable to having a $\underline{yw}$/Y; Dp^a/+ rather than an $\widehat{XY}w$/Y; +/Dp^a grandparent.

Characterization of the quantitative differences in pteridines between sons of M-11 and those of P-11 was deemed of possible value in determining whether both eye and testis sheath, which are quite different from one another in developmental origin (POULSON 1950), are affected similarly by the parental source effect. Highly significant quantitative differences in amounts of drosopterin (DP), sepia pteridine (SP) and the HB complex (biopterin and 2-amino-4-hydroxypteridine) in eyes distinguish the two types of sons, although no qualitative differences were observed (see Table 1). The amount of SP and HB (which is mainly biopterin) in the eyes bore a positive correlation, and there was less of both substances in M-11 than in P-11 sons (See Figure 2). The essentially straight line of Figure 2 leads one to infer that the parental source effect is acting on one process. However, the relation of both SP and HB to drosopterin differs in that M-11 sons show a positive correlation of both SP and HB to DP, whereas P-11 sons show negative correlation (Figures 3, 4). This may mean that at least two processes are being affected. Perhaps the M-11 sons characteristically never form enough precursors of drosopterin to make more than a small amount of visible pigment, whereas the P-11 sons accumulate excesses of the drosopterin precursors which are not converted into DP at a normal rate. The general shape of the curves in Figures 3 and 4 is reminiscent of the observation

of BAKER and SPOFFORD (1959) that the sepia and HB pteridines accumulate at intermediate values of visible pigmentation.

Highly significant differences in amounts of SP and the HB complex, as well as *iso*xanthopterin (IX), were observed also in the testis sheath, depending upon the parental source of Dp^a. Again, no qualitative differences distinguished the two kinds of sons. The relation of SP to HB was a direct positive one (Figure 5). *Iso*xanthopterin likewise showed a range of low values in M-11 sons, high values in P-11 sons (Table 1).

Within neither the $\widehat{XY}w/Y$; $Dp^a/+$ sons nor the $\widehat{XY}w/Y$; $+/Dp^a$ sons was there a correlation observed between the amount of SP of the eyes and in testis sheaths (Figure 6) nor between the amount of HB in eyes and sheaths (Figure 7). Moreover, the values for M-11 sons as a group were for both tissues lower than the values for P-11 sons. The relative lack of overlap between the two groups of points representing the two kinds of sons gives an indication of the genetic homogeneity within each group.

Direct examination of the source effect in free X males is impossible since no yw/Y; $+/Dp^a$ males from a patrilineal line were produced at the same time the M-21 matings were made. However, daughters of M-21 ($\underline{yw}/Y$; $Dp^a/+$) showed nearly identical penetrance and expression of Dp^a to that of daughters of M-11 and also daughters of MP-11, 12 and MP-21. Thus the expression of variegation in daughters appears to depend only on the source of Dp^a and not on the structure of their father's X chromosome. It seems legitimate, therefore, to compare the progeny of MP-211 ($\underline{yw}/Y$; $+/Dp^a$ ♀♀, yw/Y; $+/Dp^a$ ♂♂) with M-21 and MP-21. When 59 white-eyed sons of MP-21 were individually crossed to $\underline{yw}/Y$ females, 50 percent produced mottled progeny, establishing the fact that the fathers actually carried the duplication. The daughters of MP-211 appeared as the daughters of P-11 paternal source matings, 48.2 percent being pigmented, and the character of pigmentation was similar to the enhanced amount which develops in daughters of the patrilineal line. More sons (four percent) had pigmented eyes than ever occurred among sons of M-21 and MP-21 (zero percent). Thus it is suggested that the parental source effect of Dp^a is maintained in free X males.

The effect of added heterochromatin on pigment development of males in the Dp^a framework is drastic. The penetrance in yw/Y; $Dp^a/+$ males is at best about 30 percent of the expected 100 percent as determined by examining testes and malpighian tubules for pigment. Only progeny testing, such as was done in MP-211, reveals that Dp^a is actually present in about half of the white-eyed sons. In addition to poor penetrance of Dp^a in yw/Y males, the amount of pigment developed in such males showing variegation is markedly less than that developed in M-11 sons (see pteridine analysis, Table 1). Both kinds of sons had exactly the same kind of mother. This leads to the conclusion that additional Y heterochromatin in the genome is associated with development of far more variegated flies with more pigment in both the tissues inspected.

Modifying effects associated with Dp^l: One primary reason for distinguishing

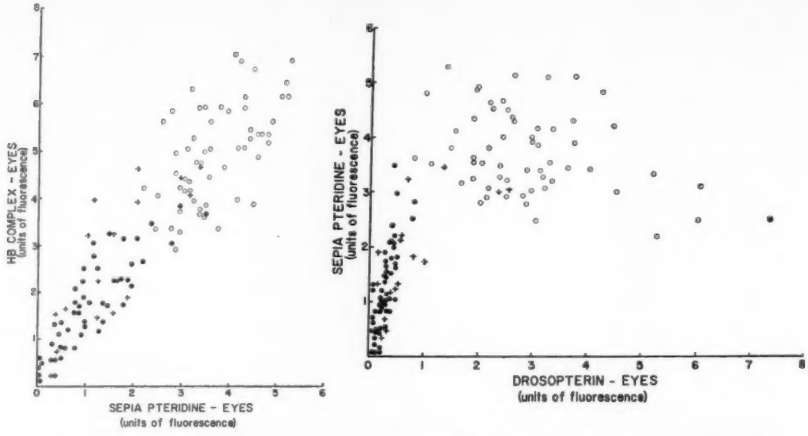


FIGURE 2.—Positive correlation of SP and $HB_1 + HB_2$ in eyes of M-11 sons ($\widehat{X}Yw/Y;Dp^a/+$, black circles), P-11 sons ($\widehat{X}Yw/Y; +/Dp^a$, open circles), and MP-13 sons ($\widehat{X}Yw/Y; Dp^a/+$, plus signs). The units of measure are arbitrary units of fluorescence as explained in Materials and Methods. These units are employed in all the figures which follow.

FIGURE 3.—Positive correlation of DP and SP in the eyes of M-11 sons ($\widehat{X}Yw/Y; Dp^a/+$, black circles); negative correlation in eyes of P-11 sons ($\widehat{X}Yw/Y; +/Dp^a$, open circles). Note intermediate values for variegated sons of $\underline{yw}/Y; Dp^a/Dp^a$, MP-13 (plus signs).

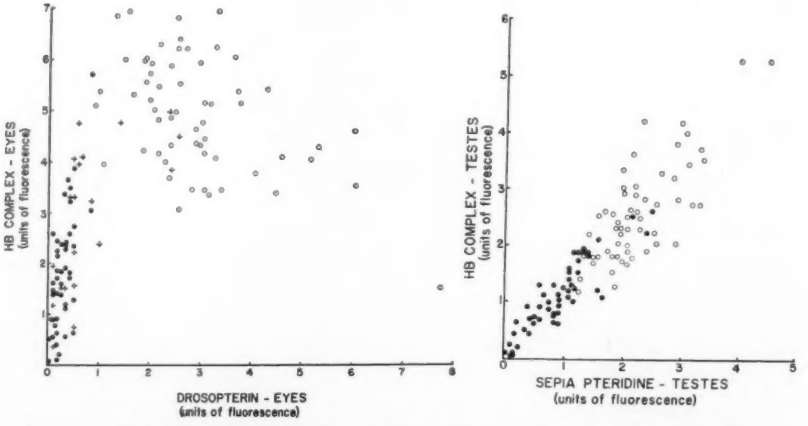


FIGURE 4.—Relation of DP to the HB complex. Symbols are the same as in Figure 3.

FIGURE 5.—Positive correlation of SP to HB complex in the testes. (Black circles = M-11 sons, $\widehat{X}Yw/Y; Dp^a/+$; open circles = P-11 sons, $\widehat{X}Yw/Y; +/Dp^a$.)

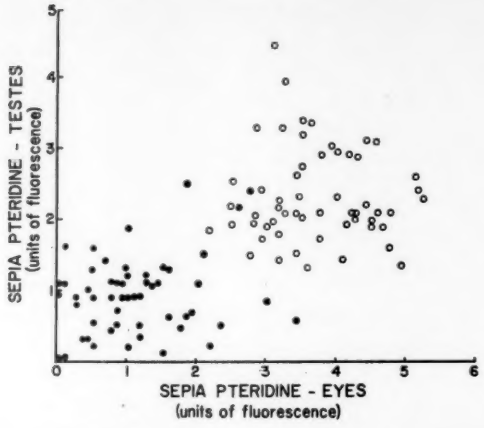


FIGURE 6.—Lack of correlation between eye and testis values of SP of M-11 sons ($\widehat{XY}w$/Y; Dp^a/+, black circles) as well as P-11 sons ($\widehat{XY}w$/Y; +/Dp^a, open circles).

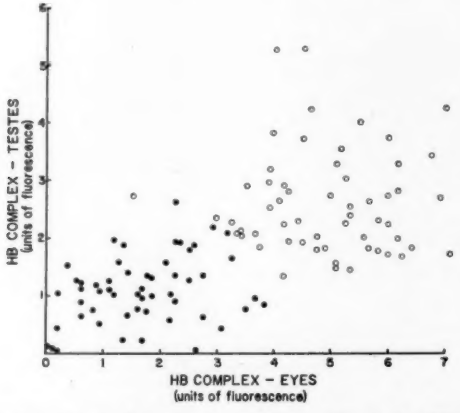


FIGURE 7.—Lack of correlation between eye and testis values of the HB complex of M-11 sons ($\widehat{XY}w$/Y; Dp^a/+, black circles) as well as P-11 sons ($\widehat{XY}w$/Y; +/Dp^a, open circles).

a Dp^a and a Dp^f state is that the parental source effect with Dp^f is either absent or, more likely, the reverse of that seen in Dp^a as described above. The main evidence for this is supplied by observations on penetrance of Dp^f in males and to some extent by chromatographic analysis. If the Dp^f parental source effect were similar to Dp^a, one would expect sons of a Dp^f mother to show equal or lower penetrance of the duplication than would sons of a Dp^f father, and the expression, in terms of pigment formation, would be significantly reduced. Also, the daughters of a Dp^f mother would be expected to show consistently lower

penetrance and expression of the duplication than the daughters of a Dp^f father.

Actually, however, mottled-eyed sons (yw/Y; Dp^f/+) of a Dp^f mother (sons of MP-22) numbered 45 percent of the total male progeny, whereas only 23 percent of the sons of a Dp^f father (mating P-21) had pigmented eyes. Although dissection of white-eyed flies for evidence of pigmented testis sheaths showed that the duplication is present in 48 percent of the sons in the latter case, similar dissection of the sons of a maternal source revealed that 58 percent had pigmented testes. Table 1 shows that the amount of drosopterin is significantly higher in MP-22 than in P-21 sons, but there is no significant difference in the amounts of SP or the HB complex, although they are slightly higher in MP-22 sons.

The variegated daughters (yw/Y; Dp^f/+) of the maternally contributed Dp^f (MP-22) number about 60 percent of the total female progeny, whereas mottled daughters of P-21 comprise 50 percent of the total female progeny. The abnormally high recovery of the Dp^f-bearing sons and daughters when the mother carries Dp^f (MP-22 and MP-232) is also unique to the Dp^f state and may be suggestive of a maternal influence of a Dp^f mother on her nonduplication bearing eggs. At any rate, it provides additional evidence supporting a distinction between Dp^a and Dp^f.

Maternal homozygosity effect: Let us first consider the matings using the Dp^a state of the duplication. The seven MP-13 matings (yw/Y; Dp^a/Dp^a ♀♀ × XYw/Y; +/+ ♂♂) were discernible because no white offspring were recovered. Homozygous females were as a rule darker than heterozygous females of the M-11 kind but not surely distinguishable from MP-11, 12 females. Progeny of MP-13 had distinctly more pigment than progeny of MP-11, 12. A chromatographic analysis of pteridines in MP-13 (XYw/Y; Dp^a/+) sons reveals higher values for all eye and testis pteridines than found in M-11 sons. The difference in drosopterin values is highly significant; the difference in HB complex values for eyes is barely significant at the five percent level; the differences between the means for all the other substances are not significant at this level. It would appear that in variegated sons, heterozygous for the duplication, from yw/Y; Dp^a/Dp^a mothers, the development of pigments is improved over that of identical sons from yw/Y; Dp^a/+ mothers.

Female progeny produced as a result of the MP-2 series of matings (which will include yw/Y; Dp^a/Dp^f females) could be of three genotypes—yw/Y; Dp^a/+, yw/Y; +/Dp^f, and yw/Y; Dp^a/Dp^f. There were in fact only two phenotypes recovered, lightly mottled females and females with a great deal more pigment, with essentially no intermediate phenotypes. A sample of 15 of the lightly mottled females and 75 darkly mottled ones were individually mated to yw/Y males; five of the 15 were fertile, while about one half of the 75 were fertile. Samples of the fertile females were studied. Ten of the darker females were identified as being Dp^a/Dp^f because they produced no white female offspring, although some of the male progeny were white-eyed; 12 dark-eyed females were heterozygous and identified tentatively as yw/Y; +/Dp^f. Five lightly mottled

females were heterozygous and identified as supposedly *yw*/Y; Dp^a/+. Thus, unlike the MP-1 matings, heterozygous daughters of MP-2 were capable of being distinguished phenotypically. The testcross series MP-21, MP-22, and MP-23 thus are of unusual interest since these crosses could confirm the differences between Dp^a and Dp^f and provide evidence of the stability of these differences.

MP-21, a mating between supposed *yw*/Y; Dp^a/+ and *yw*/Y; +/+ males produced progeny showing the penetrance and pigmentation characteristic of a maternal duplication of the Dp^a state (M-21). About 30 percent of the females were pigmented, but only slightly; all the males were white-eyed. Dissection of 96 of the males failed to reveal any with pigmented testes, but progeny testing of 59 (mating MP-221) revealed the presence of Dp^a in one half of the white-eyed males. The lack of pigment in any of the testes points to a possible difference between these sons and those of M-21.

Progeny of MP-22 were entirely different from those of MP-21. Nearly 60 percent of the females were well pigmented, a highly significant difference from the daughters of MP-21 (30 percent). Also, the male progeny (*yw*/Y; Dp^f/+) of MP-22 differed significantly from MP-21 sons (*yw*/Y; Dp^a/+) in that 45 percent of the former had pigmented eyes. A sample of the *yw*/Y; Dp^f/+ sons selected for dissection showed that 58 percent had well-pigmented testes and that considerable amounts of *iso*xanthopterin had formed.

The progeny of MP-23 individual matings of *yw*/Y; Dp^a/Dp^f × *yw*/Y; +/+ males showed a striking dichotomy in appearance of females, one half being lightly pigmented and one half more fully pigmented. Of the 168 female progeny of these ten Dp^a/Dp^f testcrosses, 90 were pair-mated to *yw*/Y; +/+ males, to check whether or not genetic evidence supported phenotypic evidence of persistence of a Dp^a:Dp^f dichotomy.

Of the 90 pair matings, 38 were fertile—26 mothers were lightly mottled, and 12 darkly mottled, thus giving an indication that the latter class was less fertile. However, since this particular set of matings was not repeated, it is not possible to say whether this difference is significant. All 38 mothers produced variegated daughters, but only the darkly mottled ones had variegated sons. Considering first the suspected Dp^a mothers, the lightly mottled ones, it is seen from the results of mating MP-231 (*yw*/Y; Dp^a/+ ♀♀ × *yw*/Y; +/+ ♂♂) that no variegated sons were produced, only white-eyed ones, and only 15.6 percent of the daughters were variegated. Although these results closely resemble those of other series of *yw*/Y; Dp^a/+ × *yw*/Y; +/+ matings, the penetrance of Dp^a in daughters of MP-231 is significantly lower (15.6 percent *vs.* 29 percent). The meaning and significance of this difference is unknown as yet, but the MP-231 results are regarded as providing strong support for the identification of these lightly mottled females as Dp^a.

The progeny of MP-232 (*yw*/Y; Dp^f/+ ♀♀ × *yw*/Y;+/+ ♂♂) resembled phenotypically those of MP-22. There is a similarity in the penetrance of Dp^f also which, although not so drastic, is consistently in the same direction of recovery of more variegated than white females (Table 1). None of the males was dissected, so whether or not more than 50 percent of the sons actually carried

Dp^f is unknown. Nevertheless, the penetrance is significantly higher than was observed in sons of P-21 where the source of Dp^f was paternal. This further supports the conclusion that the parental source effect with Dp^f does act in the direction opposite to that with Dp^a.

Among the sons of MP-23, there would be expected a *yw*/Y; Dp^a/+ and *yw*/Y; Dp^f/+ class because there is evidence of Dp^a and Dp^f daughters. However, the genotype of the white and mottled sons cannot be deduced from the phenotypic percentages (34 percent white, 66 percent mottled) since the white-eyed males which have mottled testes might represent either Dp^a or Dp^f. The fact that all the testes are variegated does, however, give confirmation of the identity of the MP-23 mothers as homozygous. Note that the expression in these sons is even higher than in the *yw*/Y; Dp^f/+ males from MP-22, indicating an enhancement of pigmentation in the sons from a mother that carried two doses of the duplication, albeit in different states.

It is stressed that the maternal effect whereby progeny of a homozygous mother show better penetrance and expression than do progeny of a heterozygous mother is a one-generation effect. Progeny testing in series MP-231 and MP-232 re-establishes the differences as observed in the MP-21 and MP-22 progeny of heterozygous mothers.

It may be of interest to introduce data on the results of crossing other daughters of MP-2 to $\widehat{XY}w$/Y males. MP-2 daughters of the two phenotypes—lightly mottled and darkly mottled—were crossed individually to $\widehat{XY}w$/Y males, and progeny of 24 fertile pairs established the existence of three groups of mothers: (1) 12 darkly mottled and homozygous for the duplication, (2) eight darkly mottled and heterozygous, and (3) four lightly mottled and heterozygous. The figures given (Table 2) are the total progeny of each of the three groups, whereas all other sets of progeny discussed in this paper were first tested for within-group heterogeneity and then pooled, if appropriate. It is of interest to compare these results with both the MP-11, 12, 13 series of crosses and the MP-21, 22, 23 series.

Let us first compare the phenotypic data in Table 2 with the results of MP-11, 12, 13. The heterozygous daughters of the MP-1 matings could not be distinguished phenotypically, whereas the heterozygous daughters of the MP-2 mat-

TABLE 2

Progeny of $\widehat{XY}$w/Y; +/+ ♂♂ × ♀♀ of three possible genotypes resulting from MP-2 cross

	Daughters			Sons		
		White-variegated			White-variegated	
Genotype of mother	White	Red	Brown-red	White	Red	Brown-red
(1) *yw*/Y;Dp^a/Dp^f darkly mottled	0	97	92	0	160	0
(2) *yw*/Y;Dp^f?/+ darkly mottled	81	74	0	103	86	0
(3) *yw*/Y;Dp^a?/+ lightly mottled	46	0	32	32	0	53

ings ($\underline{yw}$/Y; Dp^a/+ ♀♀ × yw/Y;+/Dp^f ♂♂) can be distinguished and these distinctions are supported by progeny tests to $\hat{X}Yw$/Y males. Also, the daughters of MP-13, where the mother was homozygous for Dp^a, although showing some variation as to pigmentation did not show the dichotomy of phenotypes that daughters of Dp^a/Dp^f do (Table 2). Although no measurements were made, chromatographs of some white-mottled "red" and some white-mottled "brown-red" daughters of the homozygous females (Table 2) showed much more drosopterin in the "red" group than in the "brown-red", with the latter accumulating distinctively more HB and SP. It is believed that these two phenotypic classes correspond to the darkly mottled and lightly mottled daughters, respectively, of $\underline{yw}$/Y; Dp^a/Dp^f ♀× yw/Y; +/+♂. The distinction here made between the two kinds of mottled progeny must be tentatively assigned to the contributions of the $\hat{X}Yw$/Y paternal genome *versus* that of yw/Y males, a point which was not resolved in this study. Also, the supposed $\underline{yw}$/Y; Dp^f/+ mothers recorded in Table 2 did not produce a preponderance of mottled daughters, as did the MP-22 females with which they are being compared, another difference which may be attributable to the paternal genomes. The essentially wild-type pigmentation of sons of Dp^a/Dp^f mothers (when fathers are $\hat{X}Yw$/Y) is considered very different from the measured values of MP-13 sons, which were only partly pigmented. The most important characteristic brought out by the data in Table 2 is that the daughters of MP-2 matings when crossed to $\hat{X}Yw$/Y males produce offspring which fall into three genotypic groups comparable to MP-21, 22, 23, but distinctly unlike MP-11, 12, 13.

In $\hat{X}Yw$/Y and yw/Y sons of Dp^a/Dp^f mothers there cannot be distinguished two phenotypic classes which can be said to correspond to Dp^a or Dp^f, although the female siblings in both cases do display this dichotomy, which seems to correspond genetically to Dp^a and Dp^f (MP-231, MP-232). Therefore, one must assume in the case of presumed $\hat{X}Yw$/Y; Dp^a/+ sons of Dp^a/Dp^f females that such males have their pigmentation raised to a level higher than that observed when genetically identical sons come from mothers which were homozygous for Dp^a.

Development of pigment and precursors: In order to evaluate the generality of the conclusions reached concerning pigmentation in variegated eyes and testes, a comparative study was made of the pteridines in eyes and testes of $\hat{X}Yw$/Y; +/Dp^a males and Oregon-R wild type males at a series of different times in development from two days before eclosion to 12 days after eclosion. The measurements presented in Table 3 are in terms of the arbitrary fluorescent units described earlier in the paper. These developmental studies were done on flies reared at 25±.5°C after the completion of the studies discussed previously. The relatively larger standard errors registered for the mottled males is a reflection of the more heterogeneous nature of their pigmentation compared to more uniformly pigmented wild-type males.

The amount of drosopterin in the mottled males was as a rule less than in wild type (Figure 8) whereas the amounts of sepia pteridine and the HB com-

TABLE 3

Comparison of development of eye and testis pteridines in Oregon-R and $\widehat{X}\widehat{Y}$w/Y; +/Dp^a males. Values are mean ± standard error in arbitrary fluorescent units

			Pupal stages				Imaginal stages			
Pteridine	Genotype	Age / No.	−2 days 6	−1 day 6	Eclosion 12	18±1 hr 12	2 days 16	4 days 16	9 days 17	12 days 16
Eyes										
DP	Oregon-R		.27±.01	0.74±.23	1.080±.161	4.022±.193	5.800±.153	4.756±.105	5.735±.558	4.915±.100
	$\widehat{X}\widehat{Y}w$/Y;+/Dp^a		.29±.16	1.57±.43	0.814±.131	.....	4.111±.255	2.489±.260	3.518±.202	3.875±.192
SP	Oregon-R		.30±.06	0.58±.07	0.783±.078	1.667±.076	2.133±.058	1.894±.106	1.947±.084	1.925±.051
	$\widehat{X}\widehat{Y}w$/Y;+/Dp^a		.40±.06	1.00±.11	1.107±.128	.....	2.933±.136	2.561±.136	2.606±.263	2.594±.201
HB_1 + HB_2	Oregon-R		.72±.07	0.82±.22	1.050±.083	2.289±.145	3.178±.143	3.167±.095	4.329±.181	3.885±.118
	$\widehat{X}\widehat{Y}w$/Y;+/Dp^a		.81±.09	1.73±.22	1.536±.075	.....	3.433±.179	4.339±.155	4.969±.196	5.200±.197
Testes										
SP	Oregon-R		0 or trace	0 or trace	0.288±.002	0.389±.066	0.944±.050	1.422±.075	1.059±.123	1.520±.059
	$\widehat{X}\widehat{Y}w$/Y;+/Dp^a				0.232±.002	0.867±.100	1.478±.176	2.211±.095	1.765±.074	1.850±.020
HB_1 + HB_2	Oregon-R		0 or trace	0 or trace	0.444±.002	0.767±.101	1.389±.059	1.806±.059	1.735±.050	1.660±.075
	$\widehat{X}\widehat{Y}w$/Y;+/Dp^a				0.585±.284	1.466±.186	2.056±.143	2.039±.123	2.512±.122	2.167±.106
IX	Oregon-R		0-trace	0-trace	2.422±.200	5.467±.664	14.900±.788	15.825±.476	15.040±.170	14.453±.460
	$\widehat{X}\widehat{Y}w$/Y;+/Dp^a		.33±.16	1.00±.46	2.800±.117	7.466±.917	13.600±1.335	15.210±.738	15.410±.684	14.989±.548

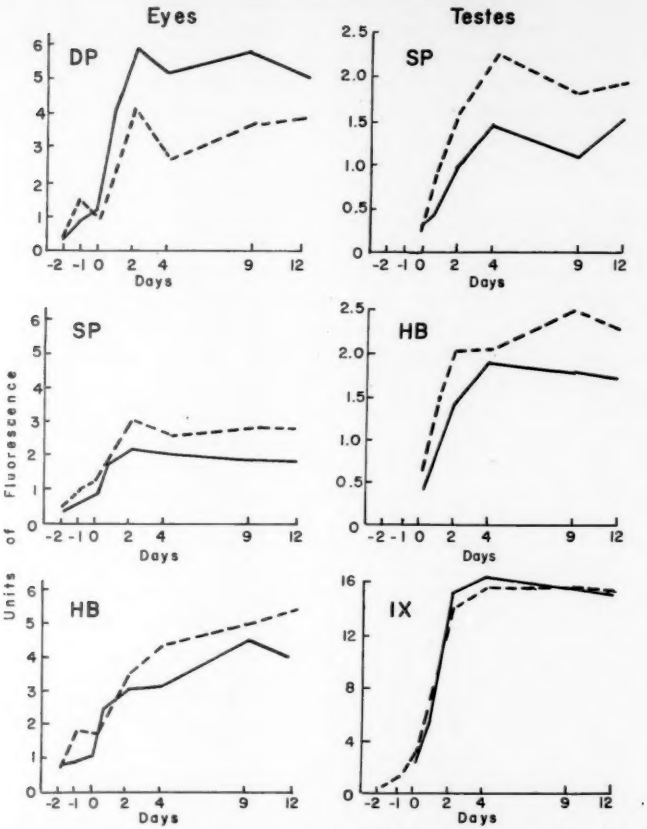


FIGURE 8.—Comparison of eye and testis pteridines developed in Oregon-R males (—) and $\widehat{XY}w/Y$; +/Dp^s males (---) which were reared concurrently. Day 0 is the time of eclosion. DP = drosopterin; SP = sepia pteridine; HB = biopterin + 2-amino-4-hydroxypteridine; IX = *iso*xanthopterin.

plex were usually higher in mottled than in wild-type males in both testes and eye tissue. If sepia pteridine is the main constituent of visible pigment in the testis sheath, it is of interest that the pigmented sheath cells of variegated testes accumulate more sepia pteridine than do the sheath cells of wild-type flies, which are completely pigmented. The *iso*xanthopterin content of the testes of the two kinds of males is essentially identical, except that the $\widehat{XY}w/Y$ males exhibit somewhat more variability. Since the synthetic pathways relating the various pteridines are not as yet known, the significance of these differences remains obscure. Apparently in males showing white-variegation, the partial blockage of red pigment formation in the eyes is accompanied by the accumulation of precursors (SP and the HB complex) in testis sheath as well as in eyes.

The values represented by the means, plotted in Figure 8, for imagoes fit very well the curves established in previous figures for 5-day-old flies. The extensive measurements made on 5-day-old flies reflect, at least in the case of $X\hat{Y}w/Y$; $+/Dp^a$ males, a difference from the wild-type condition which is characteristic throughout most of the pupal period and up to 12 days after eclosion.

DISCUSSION

Certain of the parental effects which have been described can most economically be interpreted as one-generation maternal effects. In the case of Dp^a, the progeny of a female homozygous for the duplication quantitatively express the pigment-forming action of Dp^a more strongly than do progeny of a heterozygous female. The pteridine analyses of the two sets of heterozygous sons, as well as the penetrance of Dp^a, support this statement. Implicit in such a conclusion is the assumption that one dose of Dp^a produces less pigment forming action in the egg than do two doses. Also it is possible that mutant white alleles and Dp^a are interacting such that two white alleles and one Dp^a in a heterozygous mother result in production of quantitatively less maternal pigment-forming substances than does the interaction of two white alleles with two Dp^a's in a homozygous mother. The most obvious pathway available in females for conveying maternally formed products into eggs is the transfer of nurse cell products into the developing oocyte. The egg is a closed metabolic system for the period from its fertilization until the hatching of the first larval instar. During this time (about 24 hours) differentiation is extremely rapid, and even the anlagen of imaginal gonads and eyes are laid down (POULSON 1950). Thus, there is a mechanism for the incorporation of maternal products into the cells of the imaginal anlagen. We do not know how these maternal products are maintained in the developing embryo and how they influence, eight to ten days later, the differentiation of pigment in an imaginal organ.

The other case in which a maternal effect may be tentatively invoked as an explanation of the results is the behavior of Dp^f in progeny of a maternal compared with a paternal source. It will be recalled that the progeny, heterozygous for Dp^f, produced by a Dp^f mother showed better penetrance and expression of pigmentation than progeny of a Dp^f father. This difference can be explained by assuming that the heterozygous Dp^f female adds pigment-forming substances to her eggs which are not added by white females to their eggs. Therefore, eggs ready for fertilization in a Dp^f mother would be, in a sense, conditioned for pigment development, whereas eggs of a mutant white mother would not.

Other cases of maternal influence on eggs have been noted from time to time (see CASPARI 1948). For example, in Ephestia kynurenine was demonstrated to be present in the homozygous recessive eggs (a/a) of a cross between an a^+/a♀ $\times$ a/a♂, whereas this substance is lacking in such eggs from an a/a♀ $\times$ a^+/a♂ cross. We have no knowledge of the chemical nature of the postulated substances accounting for the supposed maternal effects on white variegation in *D. melanogaster*.

In the Dp^a series, the progeny with a paternally contributed duplication exhibit

far more pigment development than do progeny of a maternal source. This is not in accord with expectations based on the postulated maternal action of the duplication. From the viewpoint of finding a consistent explanation accounting for all the phenomena observed, this is an exception which cannot be reconciled. Data are lacking which will permit critical judgment of this exception, until, for example, crosses between *yw*/Y; Dp^a/+ and $\widehat{XY}w$/Y; Dp^a/+ are made, with a marked third chromosome in either the male or the female. Thereby male progeny coming from the same type of mother and carrying either a maternally or paternally contributed Dp^a can be studied simultaneously. This study has been postponed because the addition of a newly marked third chromosome would tend to destroy the isogenicity of the stocks.

The Dp^a/Dp^f females, when crossed to either $\widehat{XY}w$/Y or *yw*/Y males produced offspring with more pigment than offspring from mothers heterozygous for either Dp^a or Dp^f. This is consistent with an explanation in terms of maternal effect. This observation implies that there is no complementation between the function of Dp^a and Dp^f insofar as the maternal homozygous effect is concerned. In other words, although these two states of the duplication may be distinguished by the particular criteria previously enumerated, they behave as functionally the same state in the maternal homozygous effect.

Since all the $\widehat{XY}w$/Y sons of both Dp^a/Dp^a and Dp^a/Dp^f mothers are pigmented, it is of interest that the sons of Dp^a/Dp^f developed nearly wild-type pigmentation whereas sons of Dp^a/Dp^a developed only partially pigmented eyes. Since the Dp^a/Dp^f female is expected to yield both Dp^a and Dp^f sons, it would appear that the Dp^a sons look like Dp^f ones. Since any egg of Dp^a/Dp^f mothers has an equal probability of developing into a male or female, potential daughters and sons would be expected to receive the same kinds and amounts of maternally contributed substances prior to fertilization. Yet the daughters of Dp^a/Dp^f show divergent phenotypes, and the sons do not. The most obvious difference between the daughters and sons is, of course, the *yw* versus the XY*w* condition; both sexes have a free Y in addition. Perhaps the association of the extra heterochromatin of XY*w* in the sons results in the passing of some crucial threshold in pigment formation which is not surpassable in the *yw* daughters. While both sons and daughters have one duplication-bearing chromosome, the sons have only one white allele, whereas the attached-X daughters have two white alleles. Whether either of these factors influences the differences observed between sons and daughters of Dp^a/Dp^f females is not known.

The fact that phenotypically distinguishable and genetically distinct Dp^a and Dp^f categories could be established certainly supports the notion that the difference in state is a stable one. The difference reported is substantiated by an independent study by DR. JANICE B. SPOFFORD (unpublished). The characteristics of the two states must represent changes in the properties of the duplication and/or surrounding heterochromatin rather than alterations caused by modifying genes (unless very tightly linked). The states could be interpreted as representing two systems of modifiers of the duplication, one enhancing (Dp^a) and the other

suppressing (Dp^f) pigment development, with crossing over in females and lack of crossing over in males to account for the parental effects described. However, since the duplication was maintained in matrilineal lines, there is always the possibility that such modifiers would eventually cross out, resulting in progressive change in the character of mottling. Such progressive alteration was not observed. The most compelling argument against two systems of modifier genes is the observation that Dp^a and Dp^f as extracted from Dp^a/Dp^f females were unchanged in their respective actions.

SUMMARY

The study disclosed the existence of two states, Dp^a and Dp^f, of the duplication $Dp(w^m)264$–58a in *Drosophila melanogaster* which produces position-effect variegation of the pigments in the eyes and testis sheaths. Dp^a flies as a rule develop less pigment than Dp^f flies when white-variegated stocks are compared. The variegated progeny heterozygous for Dp^a were more heavily pigmented if their mottled mothers were homozygous for Dp^a than if they were heterozygous for Dp^a. Variegated progeny of heterozygous Dp^a males, however, have significantly more pigmentation than do progeny of Dp^a females. Dp^f females, on the contrary, produce more heavily pigmented progeny than do Dp^f males. Also, the number of mottled progeny recovered from a $Dp^f/+$ female may be significantly higher than the number of white progeny recovered. This distorted ratio is not observed with $Dp^a/+$ mothers. The differences in penetrance and expression accompanying the parental source effects and maternal homozygosity effect in Dp^a or Dp^f are considered to be one-generation effects.

The homozygous *versus* heterozygous maternal effect is observed by comparing progeny of Dp^a/Dp^f females with progeny of either $Dp^a/+$ or $Dp^f/+$ females; thus there is no evidence for distinguishing a difference in action of Dp^a from Dp^f in terms of the homozygous maternal effect.

Chromatographic analysis of eye and testis sheath pteridines has demonstrated no correlation between sepia pteridine or $HB_1 + HB_2$ in these two tissues of $X\widehat{Y}w/Y$ males bearing Dp^a. A comparative developmental study showed that $X\widehat{Y}w/Y$; $+/Dp^a$ males as a rule developed less drosopterin in their eyes but more sepia pteridine and $HB_1 + HB_2$ in both eyes and testis sheaths than did Oregon-R wild-type males.

ACKNOWLEDGMENTS

The writer takes pleasure in acknowledging the valuable criticism and advice of Dr. William K. Baker, under whose patient sponsorship this work was accomplished. Appreciation is also expressed for the time spent in especially pleasant and stimulating discussions with Dr. Janice B. Spofford and Dr. Jack L. Hubby.

LITERATURE CITED

Albert, A., 1954 The pteridines. Fortschr. Chem. or. Naturstoffe **11**: 351–403.

Baker, W. K., and J. B. Spofford, 1959 Heterochromatic control of position-effect variegation in Drosophila. *Biological Contributions*, Univ. Texas Publ. **5914**: 135–154.

CARPENTER, J. M., 1950 A new semisynthetic food medium for Drosophila. Drosophila Inform. Serv. **24**: 96–97.

CASPARI, E., 1948 Cytoplasmic inheritance. Advances in Genet. **2**: 1–66.

DEMEREC, M., and H. SLIZYNSKA, 1937 Mottled white 258-18 of *Drosophila melanogaster*. Genetics **22**: 641–649.

FORREST, H. S., and H. K. MITCHELL, 1954a The pteridines of *Drosophila melanogaster*. pp. 143–158. *Ciba Foundation Symposium on Chemistry and Biology of Pteridines*. Little, Brown and Co., Boston.

1954b Pteridines from Drosophila. I. Isolation of a yellow pigment. J. Am. Chem. Soc. **76**: 5656–5658.

1955 Pteridines from Drosophila. III. Isolation and identification of three more pteridines. J. Am. Chem. Soc. **77**: 4865–4869.

FORREST, H. S., E. GLASSMAN, and H. K. MITCHELL, 1956 Conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in *D. melanogaster*. Science **124**: 725–726.

FORREST, H. S., D. HATFIELD, and C. VAN BAALEN, 1959 Characterization of a second yellow compound from *Drosophila melanogaster*. Nature **183**: 1269–1270.

GOWEN, J. W., and E. H. GAY, 1933 Eversporting as a function of the Y chromosome in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **19**: 122–126.

GRAF, G. E., and E. HADORN, 1959 Untersuchungen über fluoreszierende Stoffe der Hoden von Drosophila. Z. Naturforsch. **14b**: 14–17.

HADORN, E., 1959 Contribution to the physiological and biochemical genetics of pteridines and pigments in insects. Proc. 10th Intern. Congr. Genet. (1958, Montreal), 337–354.

HADORN, E., and H. K. MITCHELL, 1951 Properties of mutants of *Drosophila melanogaster* and changes during development as revealed by paper chromatography. Proc. Natl. Acad. Sci. U.S. **37**: 650–665.

LEWIS, E. B., 1950 The phenomenon of position effect. Advances in Genet. **3**: 73–115.

LINDSLEY, D. L., and E. NOVITSKI, 1959 Compound chromosomes involving the X and Y chromosomes of *Drosophila melanogaster*. Genetics **44**: 187–196.

POULSON, D. F., 1950 Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster* Meigen. *The Biology of Drosophila*. Edited by M. DEMEREC. John Wiley and Sons, Inc. New York.

SCHULTZ, J., 1936 Variegation in Drosophila and the inert chromosome regions. Proc. Natl. Acad. Sci. U.S. **22**: 27–33.

SPOFFORD, J. B., 1959 Parental control of position effect variegation. I. Parental heterochromatin and expression of the white locus in compound X *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **45**: 1003–1007.

SUTTON, E., 1940 The structure of salivary gland chromosomes of *Drosophila melanogaster* in exchanges between euchromatin and heterochromatin. Genetics **25**: 534–540.

VISCONTINI, M., 1958 Fluoreszierende Stoffe aus *Drosophila melanogaster*, 9th Mitteilung. Helv. Chim. Acta **41**: 922–925.

VISCONTINI, M., E. LOESER, and A. EGELHAAF, 1956 Oxydation des Pterins HB_2 aus Drosophila und Ephestia durch UV-Bestrahlung. Naturwiss. **16**: 379–380.

VISCONTINI, M., E. LOESER, and P. KARRER, 1958 Fluoreszierende Stoffe aus *Drosophila melanogaster*, 8th Mitteilung. Helv. Chim. Acta **41**: 440–446.

VISCONTINI, M., and H. RASCHIG, 1958 Fluoreszierende Stoffe aus *Drosophila melanogaster*. 7th Mitteilung. Helv. Chim. Acta **41**: 108.

ZIEGLER-GÜNDER, I., 1956 Pterine: Pigmente und Wirkstoffe im Tierreich. Biol. Revs. Cambridge Phil. Soc. **31**: 313–348.

ZIEGLER-GÜNDER, I., and E. HADORN, 1958 Manifestation Rezessiver Augenfarb-Gene im Pterin-inventar Heterozygoter Genotypen von *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **89**: 235–245.

GENETICS OF PHYTOPATHOGENIC FUNGI. IV. EXPERIMENTALLY INDUCED ALTERATIONS IN NUCLEAR RATIOS OF HETEROCARYONS OF FUSARIUM OXYSPORUM F. PISI[1,2]

R. W. TUVESON AND E. D. GARBER

Department of Biology, Wayne State University, Detroit 2, Michigan, and Department of Botany, University of Chicago, Chicago 37, Illinois

Received November 18, 1960

THE parasexual cycle in fungi is initiated by the formation of a heterocaryon. Consequently, genetically dissimiliar nuclei are intermingled within a common cytoplasm in which nuclear "fusion" may occur. When genotypically different haploid nuclei are involved, a heterozygous diploid nucleus results. During the multiplication of such nuclei, mitotic crossing over or haploidization or both may occur and spores may eventually be obtained which have either diploid nuclei homozygous for certain genetic markers or haploid nuclei containing new combinations of genetic markers. In either event, methods are now available for genetic studies using fungi lacking a perfect stage (PONTECORVO 1953).

BUXTON (1956) suggested that a genetic analysis of factors controlling or determining pathogenicity in the imperfect fungus *Fusarium oxysporum* f. *pisi* might be accomplished by using the parasexual cycle. TUVESON and GARBER (1959b) have shown that certain heterocaryons and diploid strains of this species yielded spores with nuclei carrying markers characteristic of only one of the component strains. These results were interpreted by assuming that selective forces favored either multiplication of one nuclear type or the incorporation of one nuclear type in the conidia. Genetics studies involving the parasexual cycle are based on the assumption that the inclusion of different nuclear types in spores is random (PONTECORVO and KAFER 1958). Situations in which certain nuclear types do not become incorporated in the spores do not lend themselves to a rational analysis.

This paper presents data indicating that it is possible to alter the presumptive selective force responsible for the anomalous situation in heterocaryons of *F. oxysporum* f. *pisi*.

MATERIALS AND METHODS

Origin of fungal stocks, cultural conditions, methods for obtaining and characterizing nutritionally deficient mutants, and for making heterocaryons have been described in detail by TUVESON and GARBER (1959a,b). Except for HET (heterocaryon) XVI, turbid spore-suspensions of each diauxotrophic strain were mixed

[1] Contribution number 64 from the Department of Biology, Wayne State University, Detroit, Michigan.

[2] This investigation was aided in part by the National Science Foundation.

and added to the surface of minimal medium plates. In preparing HET XVI, equal numbers of spores were added prior to plating the suspension.

In several experiments, the minimal medium was supplemented with the nutritional requirements of the component strain that was either not recovered or occurred in a minority of spores from the respective heterocaryons. Agar blocks containing mycelium were removed from the center of each heterocaryon actively growing on minimal medium and transferred to supplemented or unsupplemented minimal medium. Inoculated plates were incubated for 18–21 days at 26°C; spores were harvested to determine their nutritional requirements (TUVESON and GARBER 1959b).

The nutritional requirements of the eight diauxotrophic strains used in making heterocaryons were: try, his—tryptophane, histidine; nic, val—nicotinic acid, valine; lys, ade—lysine, adenine (or hypoxanthine); met, leu—methionine (or cysteine), leucine; bio, iv—biotin, isoleucine-valine; his, ade—histidine, adenine; glu, pab—glutamic acid, para-aminobenzoic acid; ino, arg—inositol, arginine.

RESULTS

The nutritional requirements of spores harvested from ten heterocaryons grown on minimal medium are presented in Table 1. The first five entries repre-

TABLE 1

Nutritional requirements of conidia harvested from heterocaryons of Fusarium oxysporum *f.* pisi *grown on minimal medium**

Heterocaryons	Component strains	Nutritional requirements
I	bio, iv–try, his	104 bio, iv
II	nic, val–his, ade	104 prototrophs
III	glu, pab–lys, ade	130 glu, pab
IV	met, leu–ino, arg	78 met, leu; 25 met; 1 leu
V	bio, iv–his, ade	104 bio, iv
VII	bio, iv–met, leu	145 bio, iv
IX	bio, iv–nic, val	98 bio, iv; 6 nic, val
XII	bio, iv–glu, pab	128 glu, pab; 1 bio, iv
XVI	his, ade–met, leu	192 his, ade
XVII	his, ade–glu, pab	78 his, ade

* HET I–V (TUVESON and GARBER 1959b).

sent data from previous experiments reported by TUVESON and GARBER (1959b) and are included for completeness. In two of the ten heterocaryons, spores with nuclei from one or the other of the component strains were recovered; in seven heterocaryons, spores had nuclei from only one component strain. In the former heterocaryons, one spore type greatly outnumbered the other one.

Spores from HET II yielded only prototrophic colonies. When this heterocaryon was stored at 5°C for six months, its spores again yielded only prototrophic colonies. TUVESON and GARBER (1959b) proposed that this anomalous heterocaryon was a diploid in which genetically unlike haploid nuclei had "fused"

during the period in which the heterocaryon was being formed. Also, it was postulated that the initial heterocaryotic mycelium which had been selected for transfer included a preponderance of diploid nuclei. To test this hypothesis, the component strains were again combined to form the heterocaryon by the same procedure. Conidia from the newly formed heterocaryon yielded 112 nic, val colonies and 103 his, ade colonies. When 1×10^5 spores from this heterocaryon were plated on minimal medium, two prototrophic colonies were obtained. These presumptive, diploids have not yet yielded spores with recombinant nuclei.

PITTENGER, KIMBALL and ATWOOD (1955) have shown that the nuclear ratio in heterocaryons of *Neurospora crassa* bears a simple relationship to the proportion of nuclei in the inoculum used to produce the heterocaryons. In HET XVI, the spores carried only the his, ade nucleus. This heterocaryon was synthesized using a spore suspension containing equal numbers of spores from each component strain. Spores harvested from this heterocaryon grown on minimal medium required histidine and adenine, indicating that the proportion of nuclear types in the inoculum did not bear any obvious relationship to the proportions obtained in conidia from the heterocaryon.

HET VII yielded only spores requiring biotin, isoleucine and valine (Table 1). It was assumed that the mycelium of this heterocaryon might include a preponderance of bio, iv nuclei needed to provide for the adequate synthesis of methionine and leucine required by nuclei of the other component strain. To test this hypothesis, the heterocaryon was grown on minimal medium supplemented with graded concentrations on methionine and leucine and the harvested spores were tested for their nutritional requirements (Table 2). The frequency of spores

TABLE 2

Nutritional requirements of conidia harvested from HET VII grown on supplemented minimal medium

Component strains	Concentration of supplements	Nutritional requirements
bio, iv– met, leu	Minimal control	104 bio, iv
	.1 mg/ml met & .2 mg/ml leu	74 bio, iv 56 met, leu
	.2 mg/ml met & .4 mg/ml leu	53 bio, iv 51 met, leu
	.3 mg/ml met & .6 mg/ml leu	27 bio, iv 75 met, leu
	.4 mg/ml met & .8 mg/ml leu	1 bio, iv 102 met, leu

met = methionine; leu = leucine.

with met, leu nuclei increased in proportion with the concentration of methionine and leucine. At the maximum concentration, spores with bio, iv nuclei had almost disappeared. These observations indicated that it was possible to alter the selective forces present in heterocaryons involving nutritionally deficient mutant strains by supplementing the minimal medium.

HET VII was grown on minimal medium supplemented with methionine or leucine to determine if both compounds were needed to alter the nuclear proportions. The concentrations of these amino acids were selected on the basis of the previous experiment so that approximately equal numbers of nuclear types could be expected. According to data in Table 3, leucine but not methionine was effective in altering nuclear ratios.

TABLE 3

Nutritional requirements of conidia harvested from HET VII grown on singly supplemented minimal medium

Component strains	Concentration of supplements	Nutritional requirements
bio, iv–	.2 mg/ml met	104 bio, iv
met, leu	.4 mg/ml leu	54 bio, iv 50 met, leu

met = methionine; leu = leucine.

Spores from HET V, grown on minimal medium, required biotin, isoleucine and valine; it was not possible to detect spores with the his, ade nucleus (Table 1). This heterocaryon was grown on minimal medium containing 0.3 mg of histidine and 0.015 mg of adenine per ml of medium for 18 days; the harvested spores yielded 75 bio, iv colonies and 55 his, ade colonies.

HET XVI included the same mutant met, leu strain involved in the previously discussed HET VII. Spores from HET XVI grown on minimal medium yielded only his, ade colonies. This heterocaryon was grown on minimal medium supplemented with the same concentrations of methionine and leucine used in the previous experiments with HET VII. The results are summarized in Table 4. At the lowest concentration, all the spores carried the formerly "minority" met, leu

TABLE 4

Nutritional requirements of conidia harvested from HET XVI grown on supplemented minimal medium

Component strains	Concentration of supplements	Nutritional requirements
his, ade– met, leu	Minimal control	104 his, ade
	.1 mg/ml met & .2 mg/ml leu	104 met, leu
	.2 mg/ml met & .4 mg/ml leu	104 met, leu
	.3 mg/ml met & .6 mg/ml leu	6 his, ade 98 met, leu
	.4 mg/ml met & .8 mg/ml leu	102 his, ade

met = methionine; leu = leucine.

nucleus. Not until three times the lowest concentration of each supplement had been added to the minimal medium was it possible to obtain spores with the his, ade nucleus. Finally, heterocaryons grown on the maximum concentration of supplements yielded only spores with the his, ade nucleus. In summary, the frequency of spores with the met, leu nucleus did not increase in proportion with increasing concentrations of methionine and leucine.

Attempts to alter nuclear ratios for spores harvested from HET III (glu, pab– lys, ade) grown on minimal medium supplemented with graded concentrations of lysine and adenine were unsuccessful. This heterocaryon continued to yield spores with the glu, pab nucleus even when the medium was supplemented with concentrations which were 15 times that required for optimal growth of the component strain requiring lysine and adenine. It is possible that this heterocaryon represents a type which is refractory to the manipulation of nuclear proportions by supplementing the medium.

DISCUSSION

Except for HET II, heterocaryons involving diauxotrophic mutant strains of *Fusarium oxysporum* f. *pisi* yielded spores bearing a nucleus from either only one or predominantly one of the component strains. It has been proposed that this situation reflected a very strong selection favoring the nuclei of one component strain in the spore-bearing mycelium of the heterocaryon. The factors determining the selected or "majority" nucleus are not immediately obvious. Rules cannot be formulated at this time to predict which nuclear component will be recovered in the spores of a particular heterocaryon. For example, HET XII yielded spores with the glu, pab nucleus; only one spore with the bio, iv nucleus was recovered. On the other hand, HET XVII yielded only spores with the his, ade nucleus and spores with the glu, pab nucleus were not discovered. In addition, notice that in HET V the bio, iv nucleus is recovered and that the his, ade nucleus was not observed. The nuclear component to be found in spores of heterocaryons of this species appears to be determined by the particular component strains used to make the heterocaryon.

According to Prout, Huebschman, Levene and Ryan (1953), the distribution of nuclei in heterocaryons of *Neurospora crassa* involving component strains with nutritional deficiencies seems to depend on the particular heterocaryon. By their method of plating conidia, it was possible to establish that both nuclear components were present in the mycelium of heterocaryons. Since heterocaryons of *F. oxysporum* f. *pisi* grown on minimal medium usually yielded spores with only one nuclear type, the proportion of nuclear types in the mycelium of heterocaryons of this species cannot be determined by plating conidia.

Pittenger and Atwood (1956) have shown that the growth rate of Neurospora heterocaryons in which the initial nuclear input ratio was relatively extreme (500:1 to 50:1) was submaximal and that there was no adaptive change in the nuclear proportions. Growth-tube experiments with these heterocaryons indicated that the nuclear ratio was relatively constant during growth. When

changes in growth did occur, they were not adaptive and the nuclei in the majority increased in frequency. BEADLE and COONRADT (1944) had postulated that the ratio of the component nuclei in heterocaryons of Neurospora was a matter of chance, providing one nuclear type had no selective advantage over the other. Furthermore, they suggested that the selected nuclear proportion in heterocaryotic mycelium resulted in optimal growth but that this ratio may have any value within very wide limits when the component nuclei were relatively "recessive".

The initial nuclear input in preparing heterocaryons of *F. oxysporum* f. *pisi* did not appear to influence the nuclear ratio of the resulting spores. Furthermore, heterocaryons seemed to yield spores with only one nuclear type relatively early during the growth of the heterocaryon and continued to do so over an extended period of time. It is possible that the hyphae closely associated with the substrate are heterocaryotic and that the aerial hyhae from which spores are presumably derived are homocaryotic for the "majority" nucleus.

RYAN and LEDERBERG (1946) investigated reverse mutation and adaptation in a leucine-requiring mutant strain of *Neurospora crassa*. Reverse mutation to prototrophy resulted in a heterocaryon. When this heterocaryon was grown on minimal medium supplemented with leucine, hyphae which originally had included some leucine-independent nuclei yielded only spores requiring this amino acid. PITTENGER and ATWOOD (1956) found that supplementation was ineffective in altering nuclear ratios in heterocaryons involving mutant strains with nutritional deficiencies. Adding the nutritional requirements of the "majority" nucleus, however, increased the growth rate of those heterocaryons exhibiting submaximal growth without altering the nuclear ratio.

JINKS (1952) investigated a heterocaryon of *Penicillium cyclopium* involving two morphological mutant strains. The heterocaryon "dissociated" on minimal medium. When the heterocaryon was transferred from an apple pulp medium to one containing minimal medium supplemented with apple pulp, there was an alteration in the nuclear ratio. As the proportion of apple pulp increased, the frequency of the "minority" nuclei also increased. According to JINKS, the component nuclei differed in their rate of multiplication and the nuclear ratio was a function of the type of medium on which the heterocaryon was grown. BUXTON (1954) reported similar observations for a heterocaryon of *F. oxysporum* f. *gladioli* involving two morphological mutant strains. Media differing in carbon concentrations or in carbon nitrogen ratios exhibited a selective effect for one or the other component nuclei, that is, the nuclear ratio was altered according to the relative concentrations of carbon or nitrogen sources in the medium.

In certain cases, nuclear ratios in heterocaryons of *F. oxysporum* f. *pisi* were determined by selective forces that could be altered by supplementing the minimal medium with the nutritional requirements of the "minority" nucleus. It is not clear at this time how supplementation accomplished this alteration of nuclear ratios.

Although HET V and HET VII exhibited an altered nuclear ratio on supplemented minimal medium, HET III was unaffected. Although the "minority"

nuclei of HET VII required methionine and leucine, only one of the amino acids (leucine) was effective in altering nuclear ratios. Whereas graded concentrations of the requirements of the "minority" nucleus common to HET XVI and HET VII were related to the nuclear output in spores from these heterocaryons, similar concentrations yielded different results for these heterocaryons. These observations clearly indicate that it is not possible to predict the outcome of supplementing the minimal medium with respect to nuclear ratios in heterocaryons of *F. oxysporum* f. *pisi*.

The effect of supplementation on nuclear ratios may be a useful tool in obtaining diploid nuclei in heterocaryons of Fusarium and, perhaps, other imperfect fungal species. The probability of a "fusion" of nuclei of different component strains would be greatly enhanced if these nuclei occurred in approximately equal numbers.

SUMMARY

In two of ten heterocaryons of *Fusarium oxysporum* f. *pisi*, involving diauxotrophic mutant strains, spores carried nuclei from both of the component strains; in seven heterocaryons, spores carried nuclei from only one of the component strains. The tenth heterocaryon yielded prototrophic spores which were presumably heterozygous diploids. When this heterocaryon was made a second time, it yielded spores with one or the other component nuclei in approximately equal numbers.

Three heterocaryons were grown on minimal medium supplemented with graded concentrations of the compounds required by the "minority" nucleus. In one heterocaryon, there was a direct relation between the concentrations and the frequency of the "minority" nucleus; in the second heterocaryon, there was no direct relationship; and in the third heterocaryon, supplementation did not alter the nuclear ratio.

The behaviour of heterocaryons of *F. oxysporum* f. *pisi* and those of other fungal species, principally Neurospora, are discussed.

ACKNOWLEDGMENT

The senior author is indebted to Wayne State University for assistance received in the form of a Faculty Research Fellowship and a Research Grant-in-aid.

LITERATURE CITED

BEADLE, G. W., and V. L. COONRADT, 1944 Heterocaryosis in *Neurospora crassa*. Genetics **29**: 291–308.

BUXTON, E. W., 1954 Heterocaryosis and variability in *Fusarium oxysporum* f. *gladioli* (Snyder and Hansen). J. Gen. Microbiol. **10**: 71–84.

1956 Heterokaryosis and parasexual recombination in pathogenic strains of *Fusarium oxysporum*. J. Gen. Microbiol. **15**: 133–139.

JINKS, J. L., 1952 Heterocaryosis in wild Penicillium. Heredity **6**: 77–87.

PITTENGER, T. H., and K. C. ATWOOD, 1956 Stability of nuclear proportions during growth of Neurospora heterocaryons. Genetics **41**: 227–241.

PITTENGER, T. H., A. W. KIMBALL, and K. C. ATWOOD, 1955 Control of nuclear ratios in Neurospora heterocaryons. Am. J. Botany **42**: 954–958.

PONTECORVO, G., 1953 The genetics of *Aspergillus nidulans*. Advances in Genet. **5**: 141–238.

PONTECORVO, G., and E. KAFER, 1958 Genetic analysis based on mitotic recombination. Advances in Genet. **9**: 71–104.

PROUT, T., C. HUEBSCHMAN, H. LEVENE, and F. J. RYAN, 1953 The proportions of nuclear types in Neurospora heterocaryons as determined by plating conidia. Genetics **38**: 518–529.

RYAN, F. J., and J. LEDERBERG, 1946 Reverse-mutation and adaptation in leucineless Neurospora. Proc. Natl. Acad. Sci. U.S. **32**: 163–173.

TUVESON, R. W., and E. D. GARBER, 1959a Genetics of phytopathogenic fungi. I. Virulence of biochemical mutants of *Fusarium oxysporum* f. *pisi*. Botan. Gaz. **121**: 69–74.

1959b Genetics of phytopathogenic fungi. II. The parasexual cycle in *Fusarium oxysporum* f. *pisi*. Botan. Gaz. **121**: 74–80.

THE GARNET LOCUS IN DROSOPHILA MELANOGASTER. I. PSEUDOALLELISM[1]

ARTHUR CHOVNICK

Biological Laboratory, Cold Spring Harbor, New York

Received November 21, 1960

PREVIOUS reports from this laboratory (CHOVNICK, LEFKOWITZ and MCQUINN 1956; CHOVNICK 1957, 1958a,b) described experiments involving assay of segregating X chromosomes from free X females heterozygous for various combinations of mutants at the garnet locus (Chromosome 1;44.4). The major observations reported may be summarized as follows:

1. From free X females heterozygous for various combinations of garnet mutants, there were recovered g^+ (nongarnet) chromosomes.
2. Such g^+ chromosomes fell into two classes: (a) Those associated with recombination for adjacent markers, and (b) A group of aberrant wild types noted for the absence of recombination for adjacent markers (or multiple recombination).
3. The recombinational wild types appear with greater frequency than the aberrant wild types in pooled data involving several mutant heterozygous combinations.

Taking into account differences in mutants used, HEXTER (1956, 1958a,b) substantially confirmed these observations using attached-X heterozygotes. However, the inferences drawn by CHOVNICK (1958a) were disputed by evidence presented by HEXTER (1958a,b), and the nature of the garnet locus left in an indecisive state (CARLSON 1959). The purpose of this report is to present more complete data leading to the inescapable conclusion that garnet is a pseudoallelic locus.

PRELIMINARY EXPERIMENTS

Females were produced which were heterozygous for all combinations of the mutants g^1, g^2, and g^{50e}. In addition, such females were heterozygous for marker genes adjacent to garnet. The markers used in this study, and their locations are shown in Diagram 1.

Assay of segregating X chromosomes of such heterozygous, free X, females was made by crossing to males with X chromosomes carrying a garnet mutant and one of the closely linked markers. In Table 1, there are recorded data collected in three groups of experiments involving an assay of 405,183 segregating X chromosomes from heterozygous females. Most of the assayed chromosomes carried a

[1] This investigation was supported by a research grant, C-4440, from the National Cancer Institute of the National Institutes of Health, Public Health Service, and by a research grant, NSF-G6431, from the National Science Foundation.

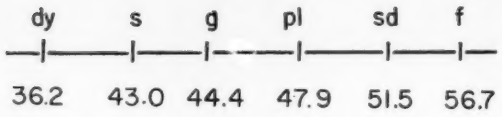


Diagram 1

dy = dusky; *s* = sable; *g* = garnet; *pl* = pleated; *sd* = scalloped; *f* = forked

TABLE 1

Assay of segregating X chromosomes from free X females of the indicated genotypes

Group		Female genotype	Nongarnet chromosomes recovered					Total chromosomes assayed
			s + *pl*	*s* + +	+ + +	*dy* + *pl*	*dy* + +	
1.	A.	*s g¹* +/+ *g² pl*	2	.	.	.	.	68,051
	B.	*dy g¹* +/+ *g² pl*	.	.	.	1	.	65,251
	C.	*dy g¹* + *sd*/+ *g² pl*+	.	.	1*	1	.	33,200
2.		*s g*^{50e}+/+ *g² pl*	2	1*	.	.	.	73,499
3.		*dy g¹ pl*/+*g*^{50e}+	.	.	2*	.	2	165,182
Totals								405,183

* Referred to in text as starred.

garnet mutant, and may, or may not have carried one or more of the closely linked markers. Twelve exceptional (nongarnet) individuals were recovered and successfully bred to verify their X chromosomal constitutions. The nongarnet chromosomes thus recovered fell into two classes. Eight possessed marker genes which indicated that they arose in association with recombination in the garnet region (nonstarred, Table 1). The remaining four nongarnet chromosomes (starred, Table 1) possessed markers indicating that their origin is either (1) not associated with recombination in the garnet region, or (2) that their origin is associated with multiple recombinational events in this region. An interpretation of these data in terms of recombination within a pseudoallelic locus ordinarily would demand that all of the exceptional chromosomes be associated with marker recombination. Restricting attention to those nongarnet chromosomes associated with marker recombination (nonstarred, Table 1), the regular behavior of marker movement is consistent with that found in pseudoallelic recombination. The recombinations of groups 1 and 2 (Table 1) place *g*^{50e} and *g¹* to the right of *g²*. The recombinations of group 3 place *g¹* to the right of *g*^{50e}. Thus, a pseudoallelic order of the garnet mutants may be inferred as shown in Diagram 2.

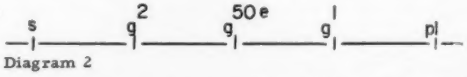


Diagram 2

Support for a pseudoallelic interpretation of the data of Table 1 comes from five sources.

A. *Evidence from studies with the mutant*, g^{3}: Introduction of a fourth mutant, *g³*, to the study may serve as a test for the pseudoallelic interpretation of the

preliminary evidence of Table 1. If we are indeed dealing with pseudoalleles, then the pattern of recombinations with g^3 should be consistent and localize g^3 in the garnet segment. In Table 2 are summarized data obtained by assaying segre-

TABLE 2

Assay of X chromosomes recovered from free X and attached-X females of the indicated genotypes

Group	Female genotype	Nongarnet chromosomes recovered from free X experiments					Total chromosomes assayed (free X expts.)	Nongarnet females recovered (attached-X expts.)
		$s+pl$	$s++$	$+++$	$dy+pl$	$dy++$		
4. A.	$s\ g^3+/+\ g^2pl$	4	1*	1*	.	.	181,102	.....
B.	$s\ g^3+/+\ g^2pl$	.	.	.	.	.	.....	4/27,770
C.	$s\ g^3sd/+\ g^2+$	.	.	.	.	.	.....	4/71,751
5. A.	$+g^3\ pl/s\ g^{50e}+$	.	1*	0	.	.	22,857	.....
B.	$+\ g^3\ pl/s\ g^{50e}+$	.	.	.	.	.	.....	3/46,760
6. A.	$+\ g^3+/dy\ g^1pl$	.	.	0	0	0	186,487	.....
B.	$s\ g^3+/dy\ g^1pl$	.	.	.	.	.	.....	0/11,388
Totals							390,446	157,669

* Starred types referred to in text.

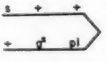
gating X chromosomes obtained from free X females, heterozygous for g^3 and each of the other garnet mutants. In addition, attached-X heterozygous females were assayed for each genetic combination. The attached-X data are summarized in Table 2 and presented in detail in Table 3. The significance of incorporating attached-X heterozygotes into the study is discussed in section D.

Attached-X heterozygotes were produced by a procedure outlined by WELSHONS (1955), utilizing the duplication, B^S. Assay for recombination within the garnet region in attached-X heterozygotes essentially follows that described by LEWIS (1952). Single attached-X females were mated, in vials, to males carrying the well-marked Muller-5 X chromosome. The parents were transferred every four days to fresh vials for a maximum of four vials/female parent. Verification of the specific genotype of each parent, with particular reference to the location of the markers was made possible by examination of the types and frequency of crossover classes.

The data of Tables 2 and 3 summarize results obtained from assaying 548,115 individuals in three categories of heterozygotes involving g^3 and g^2, g^{50e}, and g^1. There were 18 nongarnet offspring which survived to reproduce. Of these, 13 possessed nongarnet chromosomes associated with marker recombination, and five were aberrant types, not associated with marker recombination. Restricting attention to these nongarnet chromosomes associated with marker recombination, the following localization of g^3 in the garnet segment may be inferred: the group 4 recombinants (free X and attached-X) are consistent in placing g^3 to the right of g^2. The group 5, attached-X recombinants place g^3 to the right of g^{50e}. The complete failure to obtain nongarnet chromosomes from g^1/g^3 heterozygotes in

TABLE 3

Recombinations in attached-X females

	FEMALE PARENT	NON-GARNET OFFSPRING			TOTAL
		RECIPROCALS	NON-RECIPROCALS	EXCEPTIONALS	
A.		(1)	(1)	(2) 	27,770
B.		(2)	(2)		71,751
C.		(2)	(1)		46,760
TOTALS		5	4	2	146,281

both free X and attached-X females consistently places g^2 in the region of g^1. The linkage relations of the four garnet mutants thus far tested may be summarized as shown in Diagram 3.

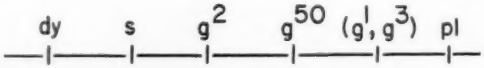


Diagram 3

B. Evidence from studies with the mutant, g^w: A fifth mutant, g^w, serves as a novel source of evidence for the pseudoallelic interpretation described above. Attempts to mark the g^w bearing chromosome with the closely linked markers, *s* (sable) and *pl* (pleated) failed repeatedly. A further linkage experiment was carried out, and the data are tabulated in Table 4. Here, it is seen that crossing

TABLE 4

Suppression of crossing over with g^w

Female genotype	Percent of recombination		No.
	v–g	*g–f*	
$\frac{v\ g\ f}{+\ +\ +}$	10.05	9.27	2685
$\frac{v\ g\ f}{+\ g^w\ +}$	3.17	0.10	2862

over in the regions *v–g–f* is suppressed drastically in the presence of g^{ic}. The mutant, *dy* (dusky) was tested against g^{ic}, and recombination was obtained (2.13 percent). These data indicate that g^{ic}, a radiation induced mutant (MULLER 1946), is associated with a rearrangement whose breaks are to the right of dusky and to the left of forked.

Cytological demonstration of the g^{ic} rearrangement is seen in Figure 1. Figure 1A shows salivary gland X chromosomes of a female carrying a wild type normal X chromosome and a chromosome marked with two large inversions, *In(1)*dl-49 and $In(1)B^{M1}$. This latter chromosome may be diagrammed as shown in Diagram 4.

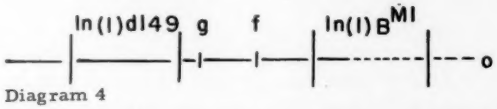


Diagram 4

The dl-49 inversion exhibits a typical inversion loop to the immediate left of the garnet region. The B^{M1} inversion, which involves the heterochromatic region between forked (*f*) and the centromere, is seen as an asynaptic region in Figure 1A.

Figure 1B shows the salivary gland X chromosomes of a female heterozygous for the g^{ic} rearrangement and the chromosome carrying *In(1)*dl-49 and $In(1)B^{M1}$. The inverted region to the right of the dl-49 inversion in this figure identifies g^{ic}. To the right of the g^{ic} inversion loop lies the asynaptic heterochromatic $In(1)B^{M1}$.

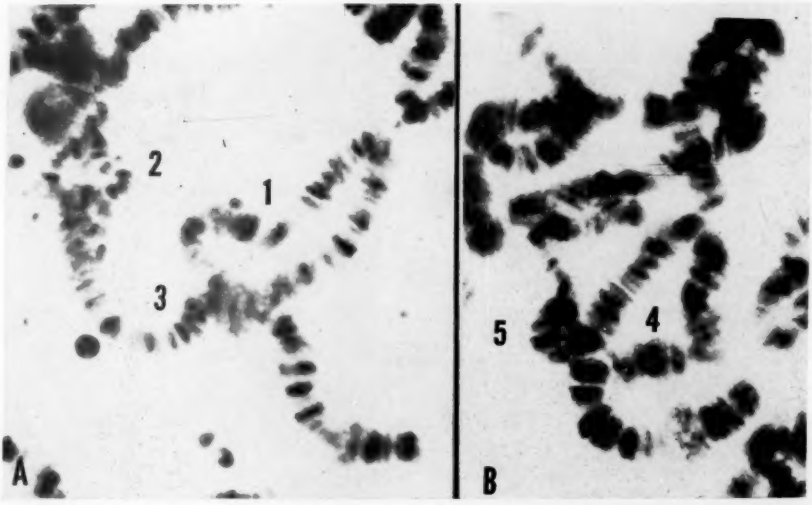


FIGURE 1A—Salivary gland X chromosomes of heterozygote, *In(1)*dl-49, *v*, g^{s1}, $In(1)B^{M1}$/Ore-R. (1) *In(1)*dl-49; (2) $In(1)B^{M1}$; (3) garnet region. FIGURE 1B—Salivary gland X chromosomes of heterozygote, *In(1)*dl-49, *v*, g^{s1}, $In(1)B^{M1}/g^{ic}$. (4) *In(1)*dl-49; (5) $In(1)g^{ic}$.

The genetic and cytological evidence presented indicates that g^{ic} is associated with an inversion leading to suppression of crossing over in the garnet region. In the prior discussion, it was inferred that the origin of nongarnet chromosomes from mutant heterozygotes is associated with crossing over within a complex locus. If this interpretation is correct, then heterozygotes of g^{ic} with all other garnet mutants should fail to yield nongarnet segregants due to the suppression of crossing over in the garnet region afforded by the inversion associated with g^{ic}. Experiments were run assaying segregating X chromosomes of heterozygotes of g^{ic} and mutants marking each end of the garnet segment (g^2 and g^3). The assay procedure was modified slightly from that used in the free X experiments described above. Heterozygous females were mated to $g^2 pl$ males, and assay of X chromosomes was restricted to the female offspring. This procedure was necessitated by the fact that g^{ic} hemizygotes and homozygotes are indistinguishable from wild type, but g^2/g^{ic} heterozygotes are clearly garnet in phenotype. In Table 5 are presented the results of these experiments. No wild type recombinants

TABLE 5

Assay of segregating X chromosomes of females of the indicated genotypes × g² pl *males*

Female genotype	X chromosomes recovered	
	g^+	g
$\frac{dy\ g^{ic}\ +}{+\ g^2\ pl}$	0	97,841
$\frac{dy\ g^{ic}\ +}{+\ g^3\ pl}$	0	78,685
Totals	0	176,526

were recovered in 176,526 X chromosomes sampled. These experiments provide further confirmation of the recombinational origin of the wild types recovered in the earlier experiments.

C. Evidence from studies with the mutant, g^{s1}: In Table 6 are summarized

TABLE 6

Assay of segregating X chromosomes from free X females heterozygous for the mutant, g^{s1}

Female genotype	Nongarnet chromosomes recovered			Total chromosomes assayed
	s ++	+++	Died without progeny	
$\frac{s\ g^{s0e}\ +}{+\ g^{s1}\ sd}$	2*	2	1	132,938
$\frac{s\ g^{s0e}\ +}{\text{dl-49},\ v,\ g^{s1},In\ B^{M1}}$	0	0	0	122,262
$\frac{dy\ g^1\ +}{+\ g^{s1}\ sd}$	0	1	0	266,614
Totals				521,814

* Starred types referred to in text.

data obtained from experiments with a sixth mutant, the spontaneous mutant, g^{s1} (SCHALET 1957). From heterozygous females, g^{50e}/g^{s1}, five nongarnet individuals were recovered in approximately 133,000 offspring. Four individuals survived to reproduce, two yielding chromosomes exhibiting marker recombination, and two were aberrant types (starred in Table 6). The recombinational types place g^{s1} to the right of g^{50e}, presumably in the cluster with g^1 and g^3. Further test of g^{s1} in heterozygotes, g^1/g^{s1}, revealed the lowest order of recombination obtained in this study (Table 6), and places g^{s1} to the right of the g^1–g^3 cluster.

Of some interest is a third experiment involving a repetition of the g^{s1}/g^{50e} test, with a slight but most significant variation. In this experiment (Table 6) the g^{s1} chromosome was marked with two large inversions, with breaks adjacent to the garnet region (on both sides), drastically reducing crossing over. In this latter experiment, it should be noted that nongarnet chromosomes failed to arise in a large sampling. This system represents the first garnet heterozygote involving g^{50e} which failed to produce both recombinational and aberrant wild types and is most clearly associated with suppression of crossing over in the garnet region.

These experiments, in addition to the earlier observations, support the notion that garnet is a pseudoallelic locus, with the order of markers as shown in diagram 5.

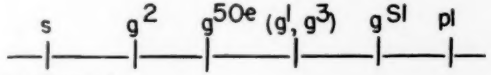


Diagram 5

D. Recombinational evidence establishing the production of multiple mutant recombination products: The data presented above lend support to the notion that nongarnet chromosomes, arising from mutant heterozygotes of garnet, are produced by a mechanism involving crossing over between adjacent sites, organized in a linear fashion, within a complex locus. Such an interpretation requires that multiple mutant chromosomes be produced in addition to the nongarnet chromosomes. Thus, if segregating X chromosomes of a pseudoallele heterozygote, $g^a+/+g^b$, are examined, then $g^a\ g^b$ double mutants should be recovered in addition to the $+\ +$ crossover product. From the free X studies, it was impossible to detect such double mutant chromosomes. It was inferred that such a chromosome was produced, but that double mutants could not be distinguished from single mutants.

In order to examine this question further, attached-X heterozygotes were studied. Methods used in the attached-X study are described in an earlier section. The major feature provided by the attached-X females is that one recovers two of the four strands involved in a meiotic event. Thus, it is possible to recover the double mutant strand (if it is produced) in the same individual possessing the nongarnet crossover product.

In Table 3 are recorded results obtained from three systems of attached-X heterozygotes that yielded nongarnet individuals. Reciprocals are those individuals possessing both of the strands involved in a crossover. Nonreciprocals are

those individuals that possess one crossover strand and one noncrossover strand. Individuals classified as exceptionals are nongarnet individuals whose chromosomes show no recombination for the closely linked markers and do not fit into the former two categories. It is of interest to note that both of the exceptionals recorded in Table 3 arose from the same parent female and may refer to a single event. The remaining nine nongarnet offspring arose independent of each other.

In Table 3, the garnet-bearing chromosome of each nongarnet attached-X female is recorded as g^x. If the reciprocals did arise by recombination between garnet mutants, then the g^x chromosome should carry the respective double mutant types. The garnet-bearing chromosome of each nonreciprocal should carry the mutant g^3 in all three systems studied if g^3 is located to the right of g^2 and g^{50e}.

From experiments with attached-X females, heterozygous for g^{50e} and g^3 (third row, Table 3), three nongarnet females were recovered. These females were mated individually to Muller-5 males, and female offspring scored for recombinations between the attached-X chromosomes. In this fashion, the presence and distribution of markers in these females were ascertained. On this basis, two of the three wild types were classed as reciprocal recombinations and should possess both g^{50e} and g^3 on the garnet-bearing chromosome. Continued mating of such females to Muller-5 males yielded a male possessing the g^x bearing chromosome, resulting from a detachment of the attached-X chromosomes in the female parent. This detached chromosome, labelled GEX-D1, served as the basis for the experiments described below.

From free X experiments described in a prior section, it was inferred that double mutants were produced, but that these were phenotypically indistinguishable from the single mutants. Observations on the garnet phenotype of the GEX-D1 chromosome are consistent with such an interpretation. Although g^{50e} and g^3 are distinguishable from each other, the garnet phenotype of the GEX-D1 chromosome is not readily separable from either mutant by microscopic examination.

If GEX-D1 does carry the double mutant, $g^{50e}g^3$, then confirmation might be attempted. Production of heterozygotes $g^{50e}\ g^3/+\ +$, and examinations for crossovers should produce a separation of g^{50e} and g^3. However, this experiment is technically not feasible due to the impossibility of distinguishing rare single mutant crossovers from the double mutant segregation product in mass cultures. An alternative experiment, based upon the following logic was carried out. If GEX-D1 does carry the double mutant, $g^{50e}\ g^3$, then assay of GEX-D1/g^3 and GEX-D1/g^{50e} should fail to yield wild type recombination products, since such females are homozygotes for g^3 and g^{50e} respectively. In contrast, GEX-D1/g^2 females should yield wild types since these females are $+\ g^{50e}\ g^3/\ g^2\ +\ +$, according to the hypothesis under consideration. The results of this experiment are summarized in Table 7. If GEX-D1 is g^{50e}, then GEX-D1/g^3 heterozygotes are expected to yield g^+ offspring with a frequency of 3.20×10^{-5} (corrected for attached-X data, Table 3), and GEX-D1/g^{50e} should yield no g^+ recombinants. Similarly, if GEX-D1 is g^3, then GEX-D1/g^{50e} should yield g^+ offspring with a

TABLE 7

Test of putative double mutant, GEX-D1. Assay of nongarnet recombination products from heterozygous females of indicated genotypes

Female parent	g^+ offspring	No.
GEX-D1/g^3	0	143,737
GEX-D1/g^{50e}	0	130,209
GEX-D1/g^2	2	39,867
Total		313,813

frequency of 3.20×10^{-5}, and no g^+ offspring should emerge from GEX-D1/g^3. As indicated in Table 7, both heterozygotes failed to yield g^+ offspring in large samplings. Utilizing a five percent Poisson confidence limit for a null event, the maximum possible frequency of g^+ production from GEX-D1/g^{50e} heterozygotes are 2.08×10^{-5} and 2.30×10^{-5} respectively. On the basis of these data, it is concluded that GEX-D1 does carry the double mutant, $g^{50e}g^3$.

Further support for this conclusion is seen from the GEX-D1/g^2 cross, which demonstrates that heterozygotes between GEX-D1 and garnet mutants at a different site are capable of yielding g^+ offspring.

E. Phenotypic identification of double mutant: Simultaneous with the experiments described in section D, another series of studies were conducted in an attempt to distinguish the eye-color phenotypes of double and single mutants using spectrophotometric examination of extracted eye pigments. Although several procedures were used to study several pigment components, this report will restrict attention to one component.

The solvent used in these studies was 30 percent aqueous ethanol acidified to *p*H-2 with HCl (EPHRUSSI and HEROLD 1944). Flies were decapitated, the mouth parts removed, and the head capsule suspended in solvent for 24 hours at 25°C in the dark. Each preparation consisted of 20 heads suspended in 3 ml of solvent. Only offspring emerging during the first three days from uncrowded cultures grown at 25°C were used in this study. Newly emerged adults were collected and aged on fresh food for two, five, and ten days at 25°C. A group of unaged flies are newly emerged adults extracted within a four-hour period following collection.

Figure 2 illustrates a typical set of data showing the UV absorption of extracts of several eye-color mutants taken from five-day aged flies. A component, with an absorption peak at 275 mμ is found, in large quantity, in the mutant *se* (sepia) as described by FORREST and MITCHELL (See Review, 1954). This component is present to a lesser degree in *v* (vermilion) and Ore-R (Oregon-R, wild type) flies, but is absent in young flies carrying the mutants *w* (white) or *bw* (brown). Small quantities of this component appear in flies of the latter two genotypes as they age.

In Figure 3, it should be noted that the 275 mμ component is affected by the garnet mutants to varying degrees. The mutant g^{w} exhibits consistently greater

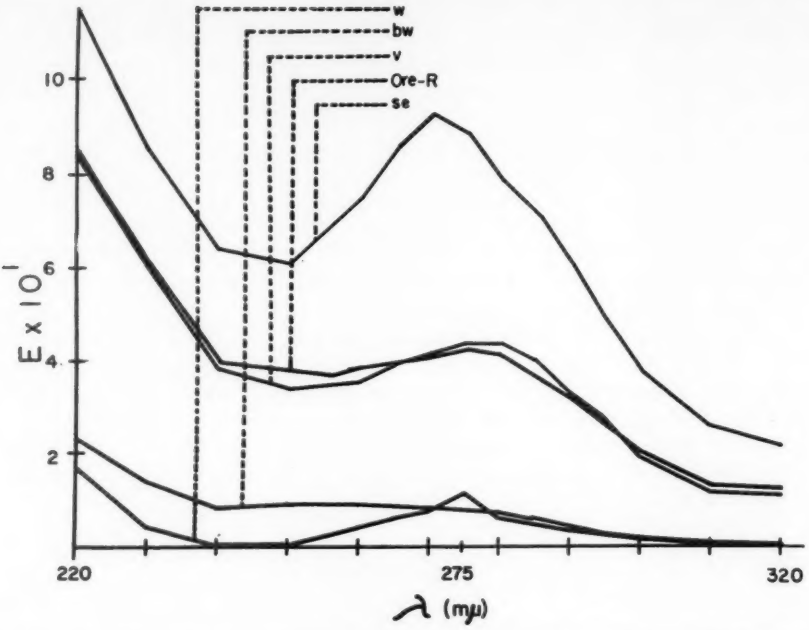


FIGURE 2—Ultraviolet absorption spectrum of extracts from five-day aged females of Oregon-R and several eye-color mutants. Abscissa: Wave length in millimicrons. Ordinate: Extinction Coefficient $\times 10^1$.

absorption at 275 mμ than either vermilion or Oregon-R. All other garnet mutants show reduction of this component as compared to Oregon-R or vermilion.

Table 8 summarizes data which provide a phenotypic distinction of the double mutant, GEX-D1 (see section D) from both single mutants g^3 and g^{50e}. It is noted that the mutants white and brown possess none of the 275 mμ component under the procedures used in this study.

An examination of the time course of development of the 275 mμ component reveals that the mutant g^{50e} starts at emergence with substantially greater quantities of this component than either g^3 or GEX-D1 (which are indistinguishable). The mutant g^3 shows increasing amounts of this component in time approaching a value near that of wild type and vermilion (Figure 3). This fact may be related to the observation that g^3 flies approach wild type as they age, and that visual separation of g^3 from wild type becomes more difficult in older flies. The ten-day readings separate g^3 from the low values seen for both g^{50e} and GEX-D1.

The experiments reported in this section, distinguishing the GEX-D1 chromosome from the single mutants g^3 and g^{50e}, support the conclusion drawn in section D that GEX-D1 does contain the double mutant $g^{50e}g^3$. Moreover, these experi-

ments (section D and E) are consistent with experiments described in earlier sections leading to the conclusion that garnet is indeed a pseudoallelic locus.

DISCUSSION

It is appropriate to attempt a reconciliation between the evidences presented in this report, establishing the pseudoallelic nature of the garnet locus, with the contradictory inference drawn by HEXTER (1958a,b). Working with three series of attached-X females, heterozygous for three different combinations of garnet mutants (and adjacent markers), HEXTER recovered nongarnet females with

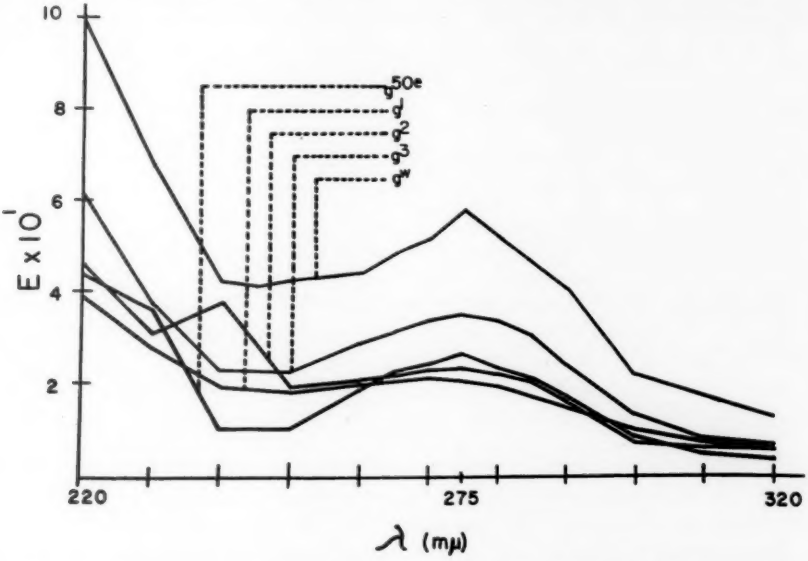


FIGURE 3—Ultraviolet absorption spectrum of extracts from five-day aged females homozygous for various garnet mutants. Abscissa: Wave length in millimicrons. Ordinate: Extinction Coefficient $\times 10^1$.

TABLE 8

Time course of development of 275 mμ component in males of indicated genotypes

Genotype	O.D.* at varying ages (days)			
	0	2	5	10
w	. . .	.000	.063	.073
bw	. . .	.058	.084	.090
g^{50e}	.272	.240	.234	.180
GEX-D1	.177	.170	.188	.140
g^2	.187	.210	.284	.420

* O.D. = Optic density

frequencies similar to those reported from this laboratory. When the distribution of adjacent markers was studied in the exceptional females, these females could be classed into the two "typical" recombination classes and a class of "exceptionals". Table 9 summarizes HEXTER's data and indicates the observed recombination frequencies (assuming pseudoallelic recombination) adjusted for attached-X females to compare with free X frequencies recorded in Table 10.

TABLE 9

*Recombination frequencies between garnet mutants adjusting for attached-X data**

Mutants tested	Adjusted recombination frequency using all wild types recovered	Adjusted recombination frequency using only "typical" recombinant classes
g^2–g^{53d}	2.54×10^{-5}	2.54×10^{-5}
g^3–g^{53d}	2.63×10^{-5}	2.10×10^{-5}
g^4–g^{53d}	3.0×10^{-5}	2.0×10^{-5}
Pooled	2.68×10^{-5}	2.23×10^{-5}

* Calculated from HEXTER (1958).

TABLE 10

*Results of tests of presumed double mutant chromosomes**

Mutant chromosomes tested	Observed recombinations	Max. frequency 5% Poisson conf. interval
$g^{53d}\ g^2$	0/ 90,104	3.33×10^{-5}
$g^{53d}\ g^3$	0/153,994	1.95×10^{-5}
$g^{53d}\ g^4$	0/115,304	2.60×10^{-5}
Pooled	0/359,403	1.67×10^{-6}

* Calculated from HEXTER (1958).

A putative double mutant chromosome was detached from each of the three systems tested, and an experiment designed to verify the double mutant nature of these chromosomes was conducted. This experiment attempts to recover one of the single mutants, g^{53d}, in further recombination experiments using free X heterozygotes of the putative double mutants. Failure to recover the single mutant was recorded in all three systems, and these data are summarized in Table 10. The maximum frequency of recombination, calculated by constructing a five percent Poisson confidence interval for a null event, is indicated for each class. Comparison of these frequencies with the observed frequencies of Table 9 reveals that two tests are insignificant, and the third is barely significant. Nevertheless, the pooled data are significant and support HEXTER's conclusion.

It is pertinent, next, to inquire into the validity of the following assumptions underlying these experiments:

(1) The frequency of recombination between a pair of closely linked pseudoalleles in a derivative double mutant chromosome is equal to the frequency of recombination between these same pseudoalleles when originally tested for recombination.

(2) In assaying male offspring of the free X pseudoallele heterozygote, $g^{53d}\ g^{x}/+\ +$, it is possible to distinguish rare $g^{53d}+$ recombination products from the $g^{53d}\ g^{x}$ nonrecombinant.

While extensive classic linkage data does support the first assumption in general, there is little data bearing on systems exhibiting such close linkage as seen in pseudoallele tests. CARLSON (1958) has collected data of some interest (Table 11). Working with the *'o²* and *'v²* pseudoalleles of dumpy, CARLSON

TABLE 11

Repeated recombination tests between pseudoalleles in cis *and* trans *derivative chromosomes*

	Percent recombination		
	'o²–'v²	*ed–cl*	No.
Cis_1	.0587	5.034	6813
Cis_2	.0377	5.240	5305
$Trans_1$	.0308	6.500	3246
$Trans_2$	.1421	4.415	11256

recovered a double mutant chromosome, subjected it to recombination tests (cis_1), then recovered both single mutants and subjected them to recombination test ($trans_1$). Once again, he extracted a double mutant chromosome which was tested (cis_2) and recovered another pair of single mutants which were tested ($trans_2$). In this experiment, recombination was scored for all markers including the outside markers *ed* (echinoid) and *cl* (clot). It should be noted that the recombination frequencies between the 'o² and 'v² pseudoalleles vary significantly, and that such variation is not due to a general effect on recombination in the region marked by the *ed* and *cl* markers. Moreover, viability differences among the different mutant combinations are excluded by comparison of the cis tests and the trans tests. Thus, in $trans_1$ and $trans_2$, the same mutant combinations score identical recombination classes, yet there is more than a fourfold difference in recombination between the pseudoalleles. Similar experiments using pseudoalleles at the white locus have been reported by ALTENBURG and ALTENBURG (1959). Although these experiments provide no critical basis for an understanding of the factors responsible for variation in recombination frequency, they do indicate, quite clearly, that caution should be exercised in assaying the significance of recombination frequency data when dealing with small distances.

With respect to the second assumption underlying HEXTER's experiment, it is suggested that while g^{53d} (orange eye phenotype) may be distinguishable from the double mutant (brownish eye phenotype) when flies of the two strains are compared, a rare g^{53d} recombinant might not be distinguishable under the conditions of this experiment. Garnet mutants studied in this laboratory show clear-cut differences from wild type (with the exception of g^{w}) and some differences among themselves. Restricting examination to young flies grown under optimal conditions, it is indeed possible to distinguish the lighter (more orange) mutants from the brownish mutants. However, the lighter mutants darken somewhat with

age and when grown under crowded conditions. Assuming that g^{53d} is clearly distinguishable from the double mutant at all ages, and when grown in crowded cultures, two further questions are of some interest: (1) If rare g^{53d} recombination products are formed, might cross-feeding play a role in altering their phenotypes sufficient to prevent distinction from the double mutant? (2) Might there be a maternal influence such that the rare g^{53d} recombinants cannot be distinguished?

In this report, emphasis is placed upon evidences leading to the conclusion that garnet is a pseudoallelic locus. In addition to the recovery of typical recombinant classes of offspring from mutant heterozygotes, chromosomes were recovered which showed no recombination (or multiple recombination) for adjacent markers. A detailed consideration of these aberrant individuals will be reported separately.

SUMMARY

In a series of experiments assaying the offspring produced by free X females, heterozygous for various mutants at the garnet locus, two classes of exceptional offspring arose: (1) a class carrying a nongarnet chromosome, associated with recombination for markers close to garnet, and (2) a class carrying a nongarnet chromosome, not associated with marker recombination (or excessive recombination).

Restricting attention in this report to the first category of offspring, evidences are presented which lead to the conclusion that garnet is a pseudoallelic or complex locus with four separable sites in the order indicated in Diagram 5.

The nature of these evidences are:

1. Consistency of marker recombination in data leading to a consistent ordering of the mutants.
2. Association of the origin of the exceptional classes with a recombinational event. Suppression of crossing over in two experiments leads to the suppression of exceptional offspring.
3. Production and confirmation of double mutant chromosomes.

An attempt is made to reconcile these data with reports by HEXTER (1958a,b) denying the pseudoallelic nature of garnet.

ACKNOWLEDGMENT

The author is indebted to DR. E. A. CARLSON of the University of California at Los Angeles for permission to include in this report a most pertinent experiment conducted in his laboratory.

LITERATURE CITED

ALTENBURG, E., and L. S. ALTENBURG, 1959 Comparative crossover rates in the *trans* and *cis* arrangements of alleles at the white locus in Drosophila. (Abstr.) Genetics **44**: 498.

CARLSON, E. A., 1958 Personal communication.

1959 Comparative genetics of complex loci. Quart. Rev. Biol. **34**: 33–67.

CHOVNICK, A., 1957 Pseudoallelism at the garnet locus in *Drosophila melanogaster* (Abstr.) Genetics **42**: 365.

1958a Aberrant segregation and pseudoallelism at the garnet locus in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **44**: 333–337.

1958b Structural and functional aspects of pseudoallelism in *Drosophila melanogaster*. (Abstr.) Proc. 10th Intern. Congr. Genet. **2**: 49.

CHOVNICK, A., R. J. LEFKOWITZ, and D. R. MCQUINN, 1956 Complexity at the garnet locus in *Drosophila melanogaster*. (Abstr.) Genetics **41**: 637.

EPHRUSSI, B., and J. L. HEROLD, 1944 Studies of eye pigments of Drosophila. I. Methods of extraction and quantitative estimation of the pigment components. Genetics **29**: 148–175.

FORREST, H. S., and H. K. MITCHELL, 1954 The pteridines of *Drosophila melanogaster*. Pp. 143–158 in *Chemistry and Biology of Pteridines*. Edited by C. E. W. WOLSTERHOLME and M. P. CAMERON. Little, Brown and Co. Boston.

HEXTER, W. M., 1956 Pseudoallelism at the garnet locus. Drosophila Inform. Serv. **30**: 121.

1958a On the nature of the garnet locus in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **44**: 768–771.

1958b Probable gene conversion in Drosophila. (Abstr.) Proc. 10th Intern. Congr. Genet. **2**: 120.

LEWIS, E. B., 1952 The pseudoallelism of white and apricot in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S. **38**: 953–961.

MULLER, H. J., 1946 New mutants of *Drosophila melanogaster*. Drosophila Inform. Serv. **20**: 66–68.

SCHALET, A., 1957 Spontaneous mutations at specific X chromosome loci in *Drosophila melanogaster*. (Abstr.) Genetics **42**: 393.

WELSHONS, W. J., 1955 A comparative study of crossing over in attached-X chromosomes of *Drosophila melanogaster*. Genetics **40**: 918–936.

VARIEGATED-TYPE POSITION EFFECTS IN THE MOUSE

LIANE BRAUCH RUSSELL AND JEAN W. BANGHAM

Biology Division, Oak Ridge National Laboratory,[1] *Oak Ridge, Tennessee*

Received December 1, 1960

UNTIL recently, V-type position effects were known only in Drosophila and Oenothera (see review by LEWIS 1950). Last year, we reported the first two cases of V-type position effect in a mammal, the mouse (RUSSELL and BANGHAM 1959). It was tentatively concluded that these were due to X-autosome translocations. Further work (RUSSELL and BANGHAM 1960) has supported this conclusion. The present paper will present the results obtained to date in experiments involving these first two cases, as well as a third one which has been investigated by us since then.

All three of the original variegated mutants arose in the course of specific locus mutation rate studies (RUSSELL 1951; RUSSELL, BANGHAM and GOWER 1958) and all were the offspring of irradiated wild-type males mated to females homozygous for seven recessive visibles (a = nonagouti, b = brown, c^{ch} = chinchilla, p = pink eye, d = dilution, se = short ear, s = piebald). As will be shown, the three cases involve variegation of the wild-type alleles at the b, c, and p loci, respectively. In two of the cases (b and p locus variegation), the mutant was sired within the first week following irradiation of the father, so that it can be assumed that the genetic change was induced in spermatozoa; in the third case (c locus variegation), the cell stage of induction may have been spermatocytes, spermatids, or spermatozoa. All three of the original variegated mutants were females which were clearly partially sterile and which transmitted variegation to their daughters.

Variegation involving the brown locus has been studied in considerably more detail than the other two cases and will be discussed first.

I. VARIEGATION INVOLVING THE *b* LOCUS

A. Progeny of variegated females; interpretation

The original mutant, on mating to a brown male, produced offspring of three types: variegated, wild type, and brown. The distribution is shown in Table 1. Almost all of the F_1 of the mutant were tested with brown mates and gave the following results: brown animals of both sexes in turn produced only brown progeny; wild-type animals produced wild-type and brown progeny in approximately equal numbers; the variegated daughters, which, it should be noted,

[1] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

were all clearly partially sterile, yielded progeny classes similar to those produced by their mother. Subsequently the stock has been maintained by continuously outcrossing variegated females to homozygous brown males. The results of all such matings are shown in Table 2.

TABLE 1

Progeny of the original brown-variegated mutant female and a homozygous brown male

	Variegated		Wild type		Brown	
	♂	♀	♂	♀	♂	♀
Fertile	—	0	1	0	4	2
Partially sterile	—	9	0	1?	0	1
Sterile	—	0	4	0	1	0
Not fertility tested	—	0	3	0	0	0
Total	0	9	8	1	5	3

TABLE 2

Offspring of brown-variegated females outcrossed to b/b *males*

	Variegated		Wild type		Brown	
	♂	♀	♂	♀	♂	♀
Fertile	—	0	3	27	9	48
Partially sterile	—	64	0	9	0	16
Sterile	—	2	12	0	2	1
Not fertility tested	—	209	263	9	325	287
Total	0	275	278	45	336	352

It should be noted that all variegated animals are females. Such animals are mottled with small brown areas irregularly interspersed with wild type. It can roughly be estimated that from 40–90 percent of the total fur is brown. Of 66 variegated females which were tested for fertility none was fully fertile and almost all were partially sterile. (The percentage of completely sterile variegated females, 2.7 percent, is not out of line with the proportion of female sterility found in many stocks of mice.) Average size of litters from the 64 partially sterile variegated females is 3.8, and the range for individual females is 1.8–5.0, with about three quarters of the females having average litter sizes in the range from 3.0 to 4.5, inclusive. By contrast, the average litter size for 32 fertile females in the stock is 7.9, with a range of 5.7–11.0. The average size of litters from the original mutant was 3.4.

Brown progeny are about evenly divided between the sexes and the bulk of those tested for fertility are fully fertile, with a few of the males sterile and some of the females partially sterile. On the other hand, the sex ratio among the wild-type progeny is very uneven. The bulk of the males tested are sterile and the bulk of the females fertile. Test matings to brown mates gave results similar to those found for the F_1 of the original mutant: brown animals of both sexes produced only browns (a total of 475); the wild-type males produced 83 brown and 87

wild-type young; and the fertile wild-type females produced 520 brown and 500 wild-type young, of which, in turn, 39 were fertile and one partially sterile. The partially sterile wild-type females will be discussed in a separate section (see section I.E).

The results discussed in this section and in sections I.B and I.C fit the interpretation which is outlined in Figure 1. According to this interpretation, variegation involves a reciprocal translocation between chromosome 8 and the X chromosome, the break in chromosome 8 being in the proximity of the *b* locus (which carries the wild-type allele). Variegated females are assumed to be heterozygous for this translocation. Their progeny may be explained as follows. (a) Unbalanced gametes do not ordinarily contribute to the surviving progeny but cause embryonic death and, thus, are the cause of the partial sterility of the variegated females. (b) Balanced gametes carrying the intact chromosomes produce fertile brown sons and daughters. (c) Balanced gametes carrying the reciprocally translocated chromosomes produce daughters that are variegated and partially sterile; and sons that, instead, are nonvariegated (i.e., wild type), and completely sterile. (In preliminary studies, OAKBERG (private communication) finds that the sterility is due to degeneration of primary spermatocytes in pachytene.) (d) Crossing over between the brown locus and the break point gives (i) wild-type young which carry the intact chromosomes and are, therefore, fertile; and (ii)

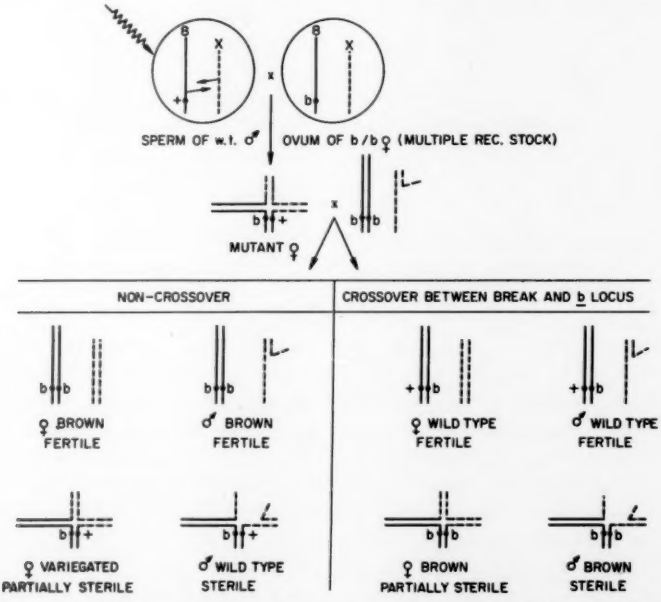


FIGURE 1.—Interpretation for the origin of brown-variegation and for the progeny of brown-variegated females crossed to *b/b* males (see text sections I. A, B, C).

brown translocation bearers, which are partially sterile, if female, and sterile, if male. Crossover frequencies will be discussed in section I.C.

Nondisjunction of the X's might be expected in appreciable frequency if the break in the X were near the centromere. This event would produce—in addition to duplication and deficiency types—the following progeny: (a) brown X0 females (intact chromosomes); (b) animals carrying the reciprocal translocation plus two intact X's, i.e., of an XXX constitution (not known whether viable, but, if so, probably variegated females); and (c) animals carrying the reciprocal translocation and of the XXY constitution. The latter would be viable males of normal size and with normal development of the external genitalia (RUSSELL and CHU 1961). By the reasoning outlined below (sec. I.E.3), they should be of variegated phenotype. Such a class has not been found; and there is additional, independent evidence (sec. I.C.2) that it is not produced with appreciable frequency, if at all. Although class (a) could be among the brown semisterile females and class (b) among the variegated females, the absence of class (c) would argue that the frequency of nondisjunction of the X is not appreciably increased by the X;8 translocation.[2]

Some evidence that animals carrying the reciprocal translocation may be slightly less viable than their nontranslocation sibs can be derived from the data of Table 2. If viability were equal, the number of wild-type males should equal the number of brown males. Actually, there are only 83 percent as many wild-type males as there are brown males and the difference from equality is significant. The bulk of the former class, of course, consists of translocation bearers, while only a relatively small proportion of the latter class is of that type (as the result of crossing over between the break point and the *b* locus). In the case of the females, the data go in the same direction but the difference is much smaller and not significant. The corresponding comparison here is that of the variegated plus wild-type classes *versus* the brown class, a ratio of 320 to 352. It is the relatively greater inviability of the male translocation bearers which accounts almost entirely for the fact that the over-all sex ratio is slightly in favor of females (614 males to 672 females).

B. Proof of variegated-type position effect

In view of the fact that we were dealing with a case unprecedented in mammals, it seemed necessary to bring formal proof that it is the *rearranged position* of the wild-type gene at the *b* locus—rather than the structural heterozygosity or a permanent change in b^+—that causes variegation. The procedure followed was modelled on that used by DUBININ and SIDOROV (1935) for hairy variegation in Drosophila. The ultimate aim of this procedure is substitution of a wild-type gene derived from a normal chromosome for the presumably rearranged wild-type gene that is variegating (see Figure 2).

In the first step, *b* was introduced into the rearrangement through crossing

[2] Note added in proof: Cattanach (Genet. Res. **2**: 156–158, 1961) has recently described XXY males in a stock carrying a presumed sex-linked rearrangement.

CROSS	FEMALE PROGENY			
	non-c.o.		c.o.	
(1) $R(b^+)/b$ ♀ × b/b ♂	$R(b^+)/b$	b/b	$R(b)/b$*	b^+/b
	v - ps	b-f	b - ps	wt-f
(2) $R(b)/b$ ♀ × $b^{+'}/b^{+'}$ ♂	$R(b)/b^{+'}$*	$b^{+'}/b$	$R(b)/b^{+'}$*	$b^{+'}/b$
	wt - ps	wt-f	wt - ps	wt-f
(3) $R(b)/b^{+'}$ ♀ × b/b ♂	$R(b)/b$	$b^{+'}/b$	$R(b^{+'})/b$	b/b
	b - ps	wt-f	v - ps	b-f

FIGURE 2.—Procedure used for proof of V-type position effect in brown-variegation. Only the female progeny of each cross is shown. Those segregants of each cross which are used in the subsequent one are marked with an asterisk. The phenotype is indicated under each genotype as follows: v = variegated, b = brown, wt = wild type; f = fertile, ps = partially sterile.

over (the required crossover class being detected through fertility tests among the brown daugters of variegated females mated to brown males). The second step was to place this rearranged *b* opposite the wild-type allele in an intact chromosome (which, in Figure 2, is designated with a prime to distinguish it from the b^+ that was originally in the rearrangement). The fact that the heterozygotes turned out to be phenotypically wild type, rather than variegated, proved that it is not structural heterozygosity that causes variegation, since the $R(b)/b^+$ and $R(b^+)/b$ types are structurally equivalent and both heterozygous for *b*. In the final step, the b^+ from the intact chromosome was introduced into the rearranged one through crossing over. Variegated, partially sterile females were again obtained, proving that it was the rearranged position of the b^+ rather than any change in the b^+ that caused the original variegation.

C. *Linkage*

1. *Linkage with the X chromosome:* The types of progeny produced by variegated females (see Tables 1, 2) give strong indications of sex linkage. More recently, it has been possible to confirm the indications with direct genetic evidence. Linkage experiments were carried out with the sex-linked, dominant marker Tabby, *Ta*, one of the few in the mouse that can be introduced through the male. Brown-variegated, heterozygous *Ta* females were produced and were mated to brown hemizygous *Ta* males. Sixteen classes were expected from such a cross, namely, the eight classes shown in Figure 1, each segregating for *Ta*. Figure 3 shows diagrammatically the alternative expectations for X independence and X linkage, respectively.

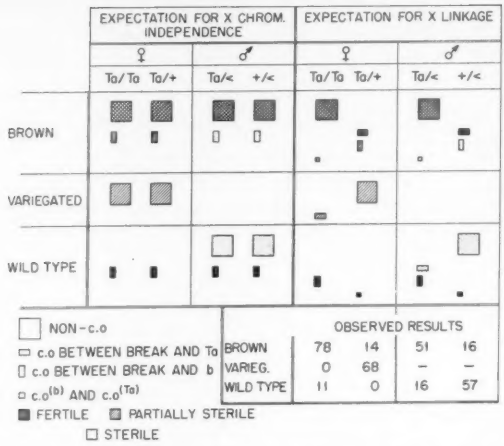


FIGURE 3.—Types of progeny from a cross of $T(b^+; Ta^+)/b$; $Ta/+$ ♀ × b/b; Ta/Y ♂: expectation for X chromosome independence and X linkage, respectively, and observed result.

If the X chromosome is not involved in the translocation, one expects equality of *Ta* segregation within each of the coat-color classes, as shown on the left side of the diagram. If, on the other hand, the translocation involves the X, one would expect the distribution shown in the right half of the diagram. Since *Ta/Ta* can be distinguished from *Ta/+* without difficulty, and *Ta/*Y from +/Y, eight classes of segregants can be classified without the further subdivision arrived at through fertility tests. The actual numbers of animals obtained in these eight classes are shown in the bottom right-hand corner of the figure. It is obvious that the bulk of the females are brown *Ta/Ta*, and variegated *Ta/+*; and the bulk of the males are brown *Ta/*Y, and wild type. This distribution is the one expected on the hypothesis of sex linkage (see right-hand portion of diagram).

It has thus been proved that brown variegation is due to a rearrangement between chromosome 8 and the X. As shown in section I.A, the assumption of a reciprocal translocation between these chromosomes fits all the data.

2. *Location of break point:* A further breakdown of the classes shown in the bottom right-hand portion of Figure 3 into fertility subclasses permits calculation of the crossover frequency between the break point and Tabby on the one hand, and the *b* locus on the other hand. The results are shown in Table 3. Crossover percentages calculated on the basis of the male progeny only are probably more accurate than those calculated on the basis of all tested animals, for the following reasons: (a) only 13 percent of the 140 males classified by coat color died before fertility test and these were distributed between crossover and noncrossover classes approximately in proportion to the totals; (b) the distinction between fertile and sterile can be made with considerably greater accuracy than the distinction between fertile and partially sterile which has to be made in the case of the females. The female data, on the other hand, are complicated in various ways

TABLE 3

Results of the cross T(b⁺;Ta⁺)/b;Ta ♀ × b/b; Ta/*Y* ♂ ; *and crossover frequencies between break point and* Ta *locus, and between break point and* b *locus calculated from the data (see Figure 3)*

Color	Females: Fertility type	Females: *Ta/Ta*	Females: *Ta/+*	Males: Fertility type	Males: *Ta/Y*	Males: *+/Y*
Brown	Fertile	45 + 24?*	5	Fertile	45	3
	Part.ster.	3	6	Sterile	0	9
Variegated	Part.ster.	0	57			
Wild type	Fertile	5 + 5?*	0	Fertile	8	0
				Sterile	6	51

Crossover frequency:	Based on: ♂♂ only	Based on: all animals
Break point and *Ta* locus	7.4%	6.3%
Break point and *b* locus	13.9%	13.2%

* Fertility type questionable due to poor general health of *Ta/Ta* (see text, sect. I.C2).

through the presence of the *Ta/Ta* classes. Thus, the relative inviability of these animals led to the death of large numbers of them (indicated by "?" in Table 3) before they had enough litters to allow an accurate decision as to their fertility type. The inclusion of 24 of these questionable animals in the brown *Ta/Ta* (noncrossover) class may—if any of them were, in effect, not wholly fertile—have led to an underestimate of crossing over. Furthermore, it should be noted that no variegated *Ta/Ta* females were found. This type may be lethal, which would also lead to an underestimate of crossing over. On the other hand, an overestimate of crossing over may be derived from the number of animals assigned to the double crossover category on the basis of their presumed partial fertility: this partial fertility may be due to the poor general health of some *Ta/Ta* females, rather than an indication of their being translocation bearers.

In spite of these various complicating factors, the crossover values calculated on the basis of all tested animals agree closely with those calculated on the basis of males only. Roughly, the frequency of crossing over between the break point and *Ta* locus is 6–7 percent, and between the break point and *b* locus 13–14 percent.

The crossing over frequency between the break point and the *b* locus can also be estimated from the larger body of data in Table 2. The genotypes of brown males and females and of wild-type males are not revealed by their coat color alone, and only relatively small proportions were fertility tested. However, by the interpretation shown in Figure 1, all wild-type females are due to crossing over and represent half of the crossover females. An estimate of crossover frequency can, therefore, be obtained from the ratio of 2× wild-type females/total

females, which gives a value of 13.4 percent, in good agreement with the 13–14 percent calculated from the independent data shown in Table 3. Because of the inclusion, in this calculation, of the problematic class of partially sterile wild-type females, which will be discussed below (sec. I.E), this figure may be a slight overestimate.

If the frequency of about 13 percent of crossing over between the break point and the *b* locus corresponds to a relatively great physical distance, then the action of the position effect must extend over a considerable length of chromosome 8. BAKER (1954) describes a V-type position effect in *D. virilis* acting over a distance (in heterochromatin) of one third the metaphase length of chromosome 5. Crossing over frequency between the break point and the affected locus was, however, low. Distances in euchromatin over which position effects in Drosophila have been found to act are quite variable (see reviews by LEWIS 1950; HANNAH 1951). In general, however, the crossover frequencies involved are smaller than in the present case of b^+ variegation in the mouse. On the other hand, a crossover frequency of almost comparable magnitude has been reported in Oenothera (CATCHESIDE 1947).

Tentative conclusions may be drawn concerning the location of the break in the X chromosome. *Ta* is relatively centrally located within the total length of 32 units that have been mapped for the X chromosome (GREEN and DICKIE 1959). If crossing over were not affected by the translocation (which, however, is unlikely), the crossing over frequency of 6–7 percent between the break point and the *Ta* locus would indicate that the break point lies either between *Mo* and *jp*, or between *Ta* and *Bn*. It is possible that future work will yield a more accurate location of the break point in the X. Similarly, it will be of interest to map the break point in chromosome 8 and to determine whether there is a spreading effect to other loci on that chromosome (e.g., misty, *m*).

The results of Table 3, unfortunately, cannot provide the answer as to whether translocation has placed the *Ta* and *b* loci on the same chromosome, or whether each translocated chromosome contains one of these loci. It should be noted that there is no obvious position effect on Ta^+: no patches of fur of the $Ta/0 = Ta/Ta$ type were noted on female translocation heterozygotes carrying *Ta* on their intact X chromosome.

The results shown in Figure 3 and Table 3 support those discussed above (sec. I.A) in giving no evidence of XXY males, a class which would be expected if the translocation had led to a high frequency of nondisjunction of the X's. Such males would be of the $X^{Ta}/X^+/Y$ constitution, which has a $Ta/+$ phenotype (RUSSELL and CHU 1961). No such animals were found.

D. Cytology

Preliminary cytological observations have been made in tissue cultures prepared from a brown-variegated, partially sterile female and from a wild-type, sterile son of a brown-variegated female.

In these two animals, 17 and 25 cells, respectively, were examined. The modal number of chromosomes was 40 (36 cells out of 42). No metacentrics were ob-

served. In almost all of the cells, one chromosome of excessive length was clearly discernible (see Figure 4). This is assumed to be one of the translocated chromosomes. Whether, corresponding to this, there is a shorter-than-normal chromosome in the complement cannot be stated since no detailed karyotype has as yet been prepared.

E. Suppression of variegation

1. *Wild-type males:* As has already been noted, male translocation heterozygotes are wild type, while females are variegated. It has been shown in Drosophila that the presence of an extra Y chromosome can suppress variegation and the removal of a Y can enhance it (see reviews by LEWIS 1950; HANNAH 1951; GRELL 1959). It was therefore considered not impossible that, in the mouse, the single Y chromosome normally present in a male might be sufficient to suppress

FIGURE 4.—Chromosomes from a spleen tissue culture preparation of a male mouse presumably heterozygous for a reciprocal translocation between chromosome 8 and the X (wild-type, sterile son of a brown-variegated female, see text, sections I. A–D). The chromosome which is of excessive length (indicated by arrow) is assumed to be one of the translocated chromosomes. × 3750.

variegation. However, more recent findings, which will be discussed in the following section (E.2), have led to a different interpretation, which will be outlined in section E.3.

2. *Partially sterile, wild-type females:* By the interpretation outlined in Figure 1, all wild-type females are expected to be fertile (with b^+ on the intact chromosome as the result of crossing over). It may, however, be seen from Table 2 that of 36 wild-type females tested for fertility, nine turned out to be only partially fertile. Three possible interpretations were considered: (a) the partially sterile wild-type females are of the expected genotype (intact chromosomes, XX) and the reduced fertility is due to some cause not connected with the translocation (e.g., random variations in fertility); (b) the partially sterile wild-type females are of some exceptional genotype but do not carry R(+); or (c) the partially sterile wild-type females carry R(+) but have some modification in their genotype which leads to suppression of variegation. Mating to brown males should produce variegated daughters only if the third explanation is correct. Actually, of the nine females in this category, only one, female 983, produced variegated daughters. Four other females had fewer than six daughters each and the results are, therefore, not conclusive for any one female by herself, although, as a group, the four females produced eight wild-type and six brown daughters. The remaining four females had ten or more daughters each, yielding a total of 25 wild type to 28 browns.

On mating the partially sterile wild-type females to Tabby males, two of them produced offspring. One of these was female 983 (who had previously thrown variegated daughters by a brown mate); the other was female 577 (who had given only nonconclusive results from a brown mate, namely, one wild-type and two brown daughters). Both of these females yielded *Ta/0* as well as *Ta/+* daughters. This result indicates that female 983 and female 577 lack either the whole intact X chromosome or an X chromosome portion containing the *Ta* locus. In the former case, the chromosome complement could, theoretically, be either 8; T(8;X); T(X;8), or 8; 8; X (with the maternal chromosome 8 carrying b^+ as a result of crossing over). However, the 8; 8; X genotype may be ruled out for both females (female 983 had variegated daughters; and female 577's wild-type sons were sterile). For the interpretation of lack of an X chromosome portion containing the *Ta* locus one may consider two possible chromosome complements: (a) X; 8; T(8;X), which is the result of the loss of one maternal chromosome and represents a deficiency for a portion of chromosome 8 (as well as portion of the X); or (b) X; 8; 8; T(X;8), which is the result of unbalanced segregation of the translocation figure in the mother and represents a duplication for a portion of chromosome 8 (as well as deficiency for portion of the X).

These various hypotheses (and subhypotheses, depending on the location of the *Ta* and *b* loci relative to each other and to the centromeres) were examined in relation to the results. First and second generation progeny of female 983 by *Ta*/Y and *Ta*/Y; *b*/*b* males fit perfectly the hypothesis that female 983 lacks the intact X. The data for female 577 are not yet as good but, so far, fit the same

hypothesis. (Under certain assumptions, more complex interpretations can also be put on these two cases.)

On the other hand, various lines of evidence indicate that three of the other seven partially sterile wild-type females probably do not lack either an entire X chromosome (chromosome count of 40) or an X chromosome portion (all of many tested daughters fully fertile). Results for the remaining four are inconclusive.

It is thus tentatively concluded that of the five partially sterile wild-type females for which some diagnostic data are available, two were probably of the chromosome constitution 8; T(8;X); T(X;8); and three may have been of the chromosome constitution 8; 8; X; X and were partially sterile for some reason not connected with the translocation.

3. *Influence of the sex chromosomes on variegation:* In view of the fact that translocation heterozygotes with sex chromosome constitutions X0 or XY are wild type, whereas those with sex chromosome constitution XX are variegated, it may be suggested that, in the mouse, two X chromosomes are required to produce variegation, and that removal of one X suppresses variegation. This is in contrast to the hypothesis mentioned in section E.1, namely, that the presence of the Y in male translocation heterozygotes might act as a suppressor of variegation. In Drosophila, it is generally true that when V-type position effects are due to rearrangement of genes from euchromatin to the vicinity of heterochromatin, variegation is markedly enhanced by the X0 condition (see reviews by LEWIS 1950 and HANNAH 1951 for specific references). The present case described for the mouse is obviously in the opposite direction.

It should be noted that one case is known in Drosophila where variegation due to rearrangement of the gene lt^+ from the proximity of heterochromatin to that of euchromatin was suppressed in the X0 male (SCHULTZ 1936). No information exists concerning heterochromatic and euchromatic regions in the chromosome complement of the mouse, and it can therefore not be stated whether the case described in this section parallels the case of the light gene in Drosophila. One respect in which parallelism fails, in any case, is in the phenotype of XY males which are wild type in R(b^+)/b mice but variegated in R(lt^+)/lt Drosophila. Furthermore, even in Drosophila, no generalizations can be based on the R(lt^+) case, since it has been shown that modification by extra Y chromosomes of the position effect at the "heterochromatic" *ci* locus is in the opposite direction from that at the *lt* locus (GRELL 1959).

If it should be true that two X chromosomes are required to produce variegation in R(b^+)/b mice, this case would parallel, in some respects, the condition found for the gene zeste, *z*, in *D. melanogaster*, where two doses of an X chromosome region including w^+ are required to produce the mottled phenotype (GANS 1953).

F. The lethal effects of the b$^+$ *rearrangement*

Preliminary data are available concerning the effect of R(b^+) in combination with lethal mutations at the *b* locus. Tests have been initiated with seven inde-

pendently induced mutations at the *b* locus, all of which act like *b* in the combinations b/b^l, and $+/b^l$, but are homozygous lethal. Brown-variegated females, $R(b^+)/b$, were mated to heterozygous brown lethal, b^+/b^l, males. If the combination $R(b^+)/b^l$ is viable, the expectation among daughters is 1 brown:2 wild type:1 variegated. The actual number found were 12:36:3. Even though these numbers are as yet small, the results already differ significantly from expectation.

Two interpretations are possible. (a) It may be assumed that the various cases of b^l represent point mutations. Under that assumption, one may conclude that the rearrangement $R(b^+)$ inactivates the b^+ allele to a recessive lethal form in a sufficiently large number of somatic cells to kill the entire animal when a nonviable allele, b^l, is present on the homologous chromosome. (b) On the other hand, if some of the b^l mutations represent deletions, rather than point mutations (and, since two of the seven were induced in postspermatogonial stages, this is a definite possibility), the lethal effect of the combination may indicate that the spreading effect extends to additional loci. That is, the rearrangement may inactivate genes adjacent to the *b* locus to a recessive lethal state in a sufficiently large number of somatic cells to cause death of the embryo when the homologous chromosome carries a deletion for the loci involved. It will be of interest to determine, when larger numbers become available, whether the different b^l mutations give different degrees of lethality in combination with $R(b^+)$.

II. VARIEGATION INVOLVING THE *c* LOCUS

Variegation of the c^+ gene has not yet been as thoroughly studied as variegation of the b^+ gene. However, the results, to date, are analogous, except for the fact that the amount of crossing over between the break point and the *c* locus appears to be considerably smaller than that between the break point and the *b* locus in the case of b^+ variegation (sec. I.C).

Offspring obtained from the outcross of variegated females to c^{ch}/c^{ch} (chinchilla) males are shown in Table 4. The following analogy to the b^+ variegation case (see Table 2) may be noted: (a) all variegated animals are females; (b) the bulk of the variegated females are partially sterile, and none is fully fertile; (c) males are of two types, namely, wild type, with all those tested, to date, sterile; and chinchilla, with all those tested, to date, fertile. The testes of the sterile males histologically resemble closely those of the $R(b^+)/b$ males (see sec. I.A) (OAKBERG, private communication).

TABLE 4

Offspring of c *variegated females outcrossed to* c^{ch}/c^{ch} *males*

	Variegated		Wild type		Chinchilla	
	♂	♀	♂	♀	♂	♀
Fertile	—	0	0	—	13	3
Partially sterile	—	27	0	—	0	0
Sterile	—	6	11	—	0	0
Not fertility-tested	—	28	50	—	78	69
Total	0	61	61	0	91	72

Animals with *c* locus variegation are mottled with diffuse patches—occasionally rather large—of the mutant tissue. When c^{ch} is carried on the intact chromosome, the mutant patches are of a color closely resembling c^{ch}/c. It thus appears that the action of the rearranged c^+ is completely suppressed to the state of the amorph, albino, in some somatic cells. It can roughly be estimated that 40–80 percent of the fur is of the mutant type, most of the animals being in the 50–60 percent range.

The main point in which the case of c^+ variegation differs from that of the case of b^+ variegation is the absence, to date, of crossing over between the break point and the *c* locus. Thus, no wild-type females have appeared; none of the fertility-tested chinchillas has been sterile; and none of the fertility-tested wild-type males has been fertile. From the data in Table 4 it may be estimated that the frequency of crossing over between the break point and the *c* locus is less than 1.5 percent.

In the case of the c^+ variegation, animals presumably heterozygous for the translocation appear to be relatively less viable than in the case of the b^+ variegation. Thus, there are only about 67 percent as many wild-type males as there are chinchilla males, and only about 85 percent as many variegated females as there are chinchilla females (roughly corresponding figures in the case of b^+ variegation are 83 percent and 91 percent, respectively). Furthermore, the c^+-variegated females are poorer producers than the b^+-variegated females: the intervals between litters are longer; a larger percentage produce no young at all; and among those that do produce young, the average litter size is smaller, namely, 2.7 (range 1.7–4.0) as compared to 3.9.

III. VARIEGATION INVOLVING THE *p* LOCUS

In the third case of presumed V-type position effect, the variegated portion of the fur is very much smaller than in the other two cases and cannot easily be characterized as to color. Variegated daughters are produced when variegated females are mated to $p\ c^{ch}/p\ c^{ch}$ (pink-eyed chinchilla) males, and the stock is maintained by continuous outcrosses of this type. Mating of variegated females to c/c (albino) males did not produce variegated daughters (in a rather limited test, but mating to p/p males did. It is, therefore, concluded that the variegating gene is p^+. (The gene *p* has a diluting effect on both the eumelanin and phaeomelanin of the coat).

Offspring produced from outcrosses of variegated females to $p\ c^{ch}/p\ c^{ch}$ males are shown in Table 5. Again, as in the other two cases (see Tables 2, 4), all variegated animals are females. The bulk of these are partially sterile (none is fertile) yielding an over-all average litter size of 2.4, with the range for individual females being 1.8–3.1. By analogy with the other two cases, the wild-type males may be assumed to represent the *male* translocation bearers. Indeed, all the members of this class that have been fertility tested to date have proved to be sterile. The testes of the sterile males histologically resemble closely those of the $R(b^+)/b$ males (see sec. I.A) (OAKBERG, private communication). The homozygous reces-

TABLE 5

Offspring of p *variegated females outcrossed to* p c^{ch}/p c^{ch} *males*

	Variegated		Wild type		$p\ c^{ch}/p\ c^{ch}$		$p\ +/p\ c^{ch}$		$+\ c^{ch}/p\ c^{ch}$	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Fertile	—	0	0	0	10	7	0	0	1	3
Partially sterile	—	16	0	0	0	0	0	0	0	0
Sterile	—	4	16	0	0	0	3	0	0	0
Not fertility-tested	—	8	30	6	36	32	4	5	2	6
Total	0	28	46	6	46	39	7	5	3	9

sive animals of both sexes, which may be assumed to represent segregants bearing the intact chromosomes, have, true to expectation, proved fully fertile (however, only 17 have been tested to date). The six wild-type females—again, by analogy with the b^+ variegation case—could represent crossovers between the break point and the *p* locus. On the other hand, in view of the fact that the variegated portions of the fur are so small in this stock, these six females might merely be normal overlaps. Breeding tests should distinguish between these alternatives.

On the hypothesis of a reciprocal translocation between chromosome 1 and the X chromosome (compare the case of b^+ variegation), there are three possible arrangements depending on the position of the break point relative to the *c* and *p* loci: (a) the break point may be *between* the loci, which would result in their being separated to two different translocated chromosomes; (b) the order may be break point, *c* locus, *p* locus; or (c) the order may be break point, *p* locus, *c* locus. It may be noted that regardless of which of these configurations exists, one expects *two* classes of variegated females: namely, in addition to the noncrossover class, which is variegated on a wild-type background, a class which is variegated either on a pink-eyed or a chinchilla background. These latter animals would represent either crossovers between the loci, or crossovers between the break point and one of the loci. The fact that such females have not yet been observed within the small $p+/p\ c^{ch}$ and $+\ c^{ch}/p\ c^{ch}$ classes may merely indicate that variegation, which is very slight in any case, cannot easily be seen on a lighter colored background. Fertility tests of the $p\ +/p\ c^{ch}$ and the $+\ c^{ch}/p\ c^{ch}$ classes, and particularly the males within these classes, should make it possible to decide between the three possible configurations of the rearrangement. Thus, in the case of configuration (b), all $p\ +/p\ c^{ch}$ males should be sterile and all $+\ c^{ch}/p\ c^{ch}$ males should be fertile; in the case of configuration (c), the situation would be the reverse; and in the case of configuration (a), both classes of males might contain a mixture of fertiles and steriles, unless crossing over between the break point and one of the loci were very infrequent or nonexistent.

DISCUSSION

For many years, V-type position effects have been known in only one animal, Drosophila, and only one plant, Oenothera. The finding of such position effects

in the mouse increases the probability that this phenomenon will eventually be demonstrated in a wide variety of species.

At the same time it should be noted that all three cases described for the mouse may represent a rather specialized mechanism, namely, one which involves sex-linked rearrangements. The number of autosome-autosome translocations is presumably a large multiple of the number of X-autosome translocations, but no V-type position effect has yet been found that does *not* involve the X. It thus appears possible that only the X has the power to produce such effects in the mouse. It is even conceivable that this power is restricted to a specific region of the X; and, in this connection, it will be important to determine whether the breakage point in the X is in the same region in all three translocations.

It is of interest that a large number of mottling mutations have been reported for the X chromosome of the mouse; and that, in fact, the bulk of sex-linked mutations of the mouse are of this type, whereas there seem to be no exactly comparable autosomal mutations. The question may, therefore, be raised whether the sex-linked mottlings are not due to some kind of position effect.

In addition to the above points, there are other features, already discussed at greater length in the paper, which distinguish the V-type position effects in the mouse from those in Drosophila. One of these distinguishing characteristics is the consistent phenotypic difference between the sexes in animals heterozygous for R(+) and the recessive: while females are variegated, males are wild type. On the basis of our finding that X0 females heterozygous for R(+) and the recessive are likewise wild type, we have suggested that two X chromosomes are required for variegation. If true, this would represent a lack of parallelism between mouse and Drosophila V-type position effects.

It should also be noted that in one of the three cases described here for the mouse, the crossover frequency between the breakage point and the variegating locus is 13–14 percent. This value is higher than crossover frequencies encountered in Drosophila position effects.

SUMMARY

1. Three cases of presumed variegated-type position effect in the mouse have been described. These involve the *b*, *c*, and *p* loci, respectively. All three were induced by irradiation in postspermatogonial stages.

2. In the case of one of these (*b* locus), the classical proof has been brought that variegation is due to a position effect produced through rearrangement of the wild-type allele.

3. The data fit the interpretation that the rearrangements are X-autosome translocations. The most direct evidence for this is the close linkage that has been found between the break point and the sex-linked marker *Ta* in the case of the b^+ variegation (c.o = 7 percent).

4. The frequency of crossing over between the variegating gene and the break point is high in the case of the b^+ variegation (13–14 percent), and low in the

case of the c^+ variegation (< 1.5 percent). No determination has yet been possible for the p^+ variegation.

5. In all three cases, the females heterozygous for the translocated wild-type gene and the recessive marker on the intact chromosome are variegated and partially sterile. Corresponding males are wild type and completely sterile (probably as a result of degeneration of primary spermatocytes in pachytene).

6. At least one female translocation heterozygote has been found which lacked the intact X. This X0 female was wild type. Since translocation heterozygotes with the sex chromosome constitution X0 or XY are wild type, while XX females are variegated, it is suggested that, in the mouse, two X chromosomes are required to produce variegation.

7. Since no case of variegated-type position effect has as yet been found which does *not* involve the X chromosome, it appears possible that only the X has the power to produce V-type position effects in the mouse. The question may also be raised whether some or all of the many sex-linked mutants that produce mottling in the mouse are not due to some kind of V-type position effect.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to DR. W. L. RUSSELL for helpful discussions; to DR. E. F. OAKBERG for histological studies of the testes of the sterile males; to DR. E. H. Y. CHU for help in the preparation of tissue cultures for cytological study and for photographing the chromosomes shown in Figure 4; and to DR. E. B. LEWIS and DR. CURT STERN for critical reading of the manuscript. We are also indebted to MRS. MARY H. STEELE and MRS. MARTHA M. LARSEN for technical assistance.

LITERATURE CITED

BAKER, W. K., 1954 The anatomy of heterochromatin. Data on the physical distance between breakage point and affected locus in a V-type position effect. J. Heredity **45**: 65–68.

CATCHESIDE, D. G., 1947 The *P*-locus position effect in Oenothera. J. Genet. **48**: 31–42.

DUBININ, N. P., and B. N. SIDOROV, 1935 The position effect of the Hairy gene. Biol. Zhur. (Mosc.) **4**: 555–568.

GANS, MADELEINE, 1953 Étude génétique et physiologique du mutant z de *Drosophila melanogaster*. Bull. Biol. France et Belg. (Suppl.) **38**: 1–90.

GREEN, MARGARET C., and MARGARET M. DICKIE, 1959 Linkage map of the mouse. J. Heredity **50**: 2–5.

GRELL, RHODA F., 1959 The Dubinin effect and the Y chromosome. Genetics **44**: 911–922.

HANNAH, ALOHA, 1951 Localization and function of heterochromatin in *Drosophila melanogaster*. Advances in Genet. **4**: 87–125.

LEWIS, E. B., 1950 The phenomenon of position effect. Advances in Genet. **3**: 73–115.

RUSSELL, LIANE B., and JEAN W. BANGHAM, 1959 Variegated-type position effects in the mouse. (Abstr.) Genetics **44**: 532.

1960 Further analysis of variegated-type position effects from X-autosome translocations in the mouse. (Abstr.) Genetics **45**: 1008–1009.

RUSSELL, LIANE B., and E. H. Y. CHU, 1961 An XXY male in the mouse. Proc. Natl. Acad. Sci. U. S. **47**: 571–575.

RUSSELL, W. L., 1951 X-ray-induced mutations in mice. Cold Spring Harbor Symposia Quant. Biol. **16**: 327–336.

RUSSELL, W. L., JEAN W. BANGHAM, and JOSEPHINE S. GOWER, 1958 Comparison between mutations induced in spermatogonial and postspermatogonial stages in the mouse. Proc. 10th Intern. Congr. Genet. **2**: 245–246.

SCHULTZ, JACK, 1936 Variegation in Drosophila and the inert chromosome regions. Proc. Natl. Acad. Sci. U.S. **22**: 27–33.

CHANGES OF MEAN FITNESS IN RANDOM MATING POPULATIONS WHEN EPISTASIS AND LINKAGE ARE PRESENT[1]

KEN-ICHI KOJIMA AND THERESE M. KELLEHER

Department of Genetics, North Carolina State College

Received December 12, 1960

IN 1930 R. A. FISHER formulated the fundamental theorem of natural selection which states that "the rate of increase in (mean) fitness of any organism at any time is equal to its genetic variance in fitness at that time" (FISHER 1930). Later, some modifications and variations of this theorem were considered by WRIGHT (1949, 1955), CROW and KIMURA (1956) and KIMURA (1958) in attempts to interpret the theorem over wider genetic circumstances.

When the members of a population are mating at random and their fitness values do not vary over time, FISHER's theorem is usually understood to imply that the mean fitness will *always increase* with time and that this increase is equal to the additive genetic variance in fitness. In multiple-locus systems, however, such interpretations of the theorem may lead one to an incorrect prediction of population behavior. With nonallelic interactions and linkages of genes the rate of change in mean fitness will be, in general, different from the additive genetic variance in fitness. In some cases the mean fitness may actually decrease during the course of evolution despite natural selection.

The objectives of this paper are to investigate the contributions to the change in mean fitness made by epistatic genes and to evaluate the effects of linkage complication among epistatic genes on such contributions. Some attempts will be made to analyze the genetic variance in terms of the partitions of genotypic variance associated with epistatic gene action. In order to avoid further complications, constancy of fitness values, random mating populations and two alleles per locus are assumed.

The change of mean fitness in a two-locus system

When genes at two loci, *A-a* and *B-b*, are segregating in a random mating population, nine different genotypes are present, and their fitness values can be assigned as in Table 1. At meiosis such a population produces four different gametes, *AB*, *Ab*, *aB* and *ab* with the frequencies, x, y, z and u, respectively. Obviously these frequencies must satisfy the condition, $x + y + z + u = 1$. The gene frequencies are $p = x + y$ for gene *A*, $(1 - p) = z + u$ for gene *a*, $q = x + z$

[1] Contribution No. 1249 of the Journal Series. North Carolina Agricultural Experimental Station. This work has been carried out under Contract No. AT-(40-1)-2798 U.S. Atomic Energy Commission, and also supported by a grant from the National Science Foundation.

TABLE 1

The fitness values of all possible genotypic arrays with two loci

Locus *A* / Locus *B*	*AA*	*Aa*	*aa*
BB	W_{22}	W_{12}	W_{02}
Bb	W_{21}	W_{11}	W_{01}
bb	W_{20}	W_{10}	W_{00}

for gene B and $(1-q)=y+u$ for gene b. After many generations of random mating the gametic frequencies will satisfy the following relations if evolutionary forces are absent:

$$x=pq, \qquad y=p(1-q)$$
$$z=(1-p)q, \quad u=(1-p)(1-q)$$

Such a state is called *linkage equilibrium*. In general, the population is not in a state of linkage equilibrium and the deviation from the equilibrium can be measured by $D=xu-yz$, i.e., the difference betweeen the product of the frequencies of coupling gametes and that of repulsion gametes.

The primary effects of natural selection due to differential values in fitness can be expressed as the rates of change in gametic frequencies at a given time, t. Using Malthusian parameters for the measure of fitness (W), KIMURA (1956) formulated these rates as follows:

$$\begin{aligned}
\frac{dx}{dt} &= x(W_x-\overline{W})-rW_{11}D \\
\frac{dy}{dt} &= y(W_y-\overline{W})-rW_{11}D \\
\frac{dz}{dt} &= z(W_z-\overline{W})-rW_{11}D \\
\frac{du}{dt} &= u(W_u-\overline{W})-rW_{11}D
\end{aligned} \qquad [1]$$

where r is the recombination fraction between the two loci, and W_x, W_y, W_z, and W_u are the marginal mean fitnesses of the gametes, *AB*, *Ab*, *aB* and *ab*, respectively. By virtue of random mating they are easily obtained from Table 2.

The mean fitness of the population, $\overline{W}$, is written as

$$\overline{W}=xW_x+yW_y+zW_z+uW_u \qquad [2]$$

Then, the rate of change in $\overline{W}$ with respect to time, t, is

$$\frac{d\overline{W}}{dt}=\frac{dx}{dt}W_x+\frac{dy}{dt}W_y+\frac{dz}{dt}W_z+\frac{du}{dt}W_u+x\frac{dW_x}{dt}+y\frac{dW_y}{dt}+z\frac{dW_z}{dt}+u\frac{dW_u}{dt} \qquad [3]$$

The derivatives of the marginal means such as $\frac{dW_x}{dt}$ can be written in terms of $\frac{dx}{dt}$, $\frac{dy}{dt}$, $\frac{dz}{dt}$ and $\frac{du}{dt}$ and formula, [3], becomes

$$\frac{d\overline{W}}{dt}=2\left(W_x\frac{dx}{dt}+W_y\frac{dy}{dt}+W_z\frac{dz}{dt}+W_u\frac{du}{dt}\right) \qquad [4]$$

Since the sum of the rates given in [1] is zero, this sum multiplied by $2\overline{W}$ can be subtracted from the right-hand side of [4] without change in $d\overline{W}/dt$. Then the result is

$$2\left[(W_x-\overline{W})\frac{dx}{dt}+(W_y-\overline{W})\frac{dy}{dt}+(W_z-\overline{W})\frac{dz}{dt}+(W_u-\overline{W})\frac{du}{dt}\right]$$

Substituting $\frac{dx}{dt}$, $\frac{dy}{dt}$, $\frac{dz}{dt}$ and $\frac{du}{dt}$ given in [1] into the last expression, the rate of change in mean fitness is obtained as

$$\frac{d\overline{W}}{dt}=2\left[x(W_x-\overline{W})^2+y(W_y-\overline{W})^2+z(W_z-\overline{W})^2+u(W_u-\overline{W})^2\right]$$
$$-2(W_x-W_y-W_z+W_u)W_{11}rD \qquad [5]$$

It is clear that the first term in [5] is twice the variance among the gametic marginal means. Let this variance be represented by V_m which may be called the marginal variance. Further let α_{AB} stand for $(W_x-W_y-W_z+W_u)$. Then the rate of change in mean fitness is written simply as $2V_m-2\alpha_{AB}W_{11}rD$. The first term is always positive, but the second term can be positive or negative, depending upon the signs of α_{AB} and D.

If D is positive, there is an excess of coupling gametes over repulsion ones. Now suppose that the repulsion gametes are more fit than the coupling gametes on the average, then the sign of α_{AB} is negative. The product $-\alpha_{AB}D$ is positive, and the result is that the rate of change in mean fitness will be inflated from the rate given by the marginal variance. This is the reflection of the situation that when

TABLE 2

The mating of gametes, fitness values and frequencies of zygotes with two loci

Female gametes	Male gametes	*AB* x	*Ab* y	*aB* z	*ab* u	Means
AB	x	W_{22} x^2	W_{21} xy	W_{12} xz	W_{11} xu	W_x
Ab	y	W_{21} xy	W_{20} y^2	W_{11} yz	W_{10} yu	W_y
aB	z	W_{12} xz	W_{11} yz	W_{02} z^2	W_{01} zu	W_z
ab	u	W_{11} xu	W_{10} yu	W_{01} zu	W_{00} u^2	W_u
Means		W_x	W_y	W_z	W_u	$\overline{W}$

the less fit types are more frequent than would be expected in a final equilibrium population, the process of selection against such types is accelerated. On the other hand, suppose that the coupling gametes are more fit than the repulsion types in a population with an excess of coupling gametes. Then $-\alpha_{AB}D$ is negative and the rate of change in mean fitness is deflated. This reflects the situation that with an excess of the better gametes the pressure toward a final equilibrium is lessened. The interpretation of formula [5] for the cases with negative D can be made in similar fashion.

An extreme deflation effect may result in an over-all decrease (instead of increase) in mean fitness, if the linkage disequilibrium with an excess of gametic types which are, on the average, more fit is so large that the positive contribution from the marginal variance is overcome by the deflation effect. Such an instantaneous decrease in mean fitness is an interesting exception to the fundamental theorem of natural selection. A numerical example of such cases will be found in the following section (Matrix 2, $D = -0.15$ and $r = 0.5$ in Table 5). An important question in this connection is whether it is possible for continuously evolving systems to build up a high degree of linkage disequilibrium which makes the magnitude of $2\alpha_{AB}W_{11}rD$ larger than the value of $2V_m$. At present there seems to be no easy way to give the answer to this question. In the work by LEWONTIN and KOJIMA (1960), however, they pointed out that the values of D could change rather drastically over a wide range (the maximum range being ± 0.25) in epistatic systems under natural selection, and their method could be used to study this problem numerically.

The decrease in mean fitness may only be possible in some special instances. One such instance is expected when two or more previously isolated populations are intercrossed, and a breakdown of a high degree of linkage disequilibrium takes place in the presence of previously adapted epistatic complexes. It is not known for how long a period of time this decrease can continue. Experimentally observations of this nature are reported by VETUKHIV (1953), KING (1955) and a few others in the studies of so-called "fitness component characters."

Finally one should point out the similarity between equation [5] and WRIGHT's equation, $\frac{d\bar{W}}{dq} = \Sigma W\frac{df}{dq} + \Sigma f\frac{dW}{dq}$, in which f stands for genotypic frequencies and the W's are frequency dependent selective values of genotypes at a single locus (WRIGHT 1949; LI 1955). If individual gametes in the linkage-epistasis systems are considered as multiple alleles at a locus with frequency dependent selective values, WRIGHT's equation and equation [5] are equivalent. LEWONTIN (1958) showed that it is possible to have stable equilibria at the points of nonmaximum mean fitness in the system with frequency dependent selective values. In turn, this indicates that sometimes during the evolution of the population the rate of change in mean fitness can be negative. Such a case is in accordance with the conclusion of decreasing mean fitness drawn from equation [5].

Partitions of marginal variance

An examination of Table 2 shows that the marginal variance, V_m, is composed

of three components. One component is associated with the comparison, (*AB* and *Ab*) vs. (*aB* and *ab*), the second with (*AB* and *aB*) vs. (*Ab* and *ab*) and the third with (*AB* and *ab*) vs. (*Ab* and *aB*). In terms of the marginal means, they are

(a) $\alpha_A = (z+u)(xW_x + yW_y) - (x+y)(zW_z + uW_u)$
(b) $\alpha_B = (y+u)(xW_x + zW_z) - (x+z)(yW_y + uW_u)$
(c) $\alpha_{AB} = (W_x - W_y - W_z + W_u)$

where α_A, α_B and α_{AB} are the additive comparisons at locus *A* and at locus *B*, and the additive × additive comparison from both loci, respectively. One of the crucial problems in constructing V_m from these comparisons is that α_A, α_B and α_{AB} are not orthogonal to each other as long as *D* is not zero.

The weights which give rise to a set of three orthogonal comparisons among the four gametic marginal means are found in Table 3. From these weights the three orthogonal components are computed as follows:

TABLE 3

Orthogonal weights for partitioning the marginal variance

Gametes	*AB*	*Ab*	*aB*	*ab*
Frequencies	x	y	z	u
Marginal means	W_x	W_y	W_z	W_u
α_A	$(z+u)$	$(z+u)$	$-(x+y)$	$-(x+y)$
α_B	$y(z+u)$	$-x(z+u)$	$u(x+y)$	$-z(x+y)$
α_{AB}	$\frac{1}{x}$	$-\frac{1}{y}$	$-\frac{1}{z}$	$\frac{1}{u}$

(a) additive component at *A*; σ_A^2: $\dfrac{{\alpha_A}^2}{p(1-p)}$

(b) additive component at *B* adjusted for α_A; σ_B^2: $\dfrac{\left[\alpha_B - \dfrac{D\alpha_A}{p(1-p)}\right]^2}{q(1-q) - \dfrac{D^2}{p(1-p)}}$ [6]

(c) additive × additive at *A* and *B*; σ_{AB}^2: $\dfrac{xyzu\,{\alpha_{AB}}^2}{p(1-p)q(1-q) - D^2}$

where $x = pq + D$, $y = p(1-q) - D$, $z = (1-p)q - D$ and $u = (1-p) \times (1-q) + D$. The sum of the two components, σ_A^2 and σ_B^2 amounts to one half of the total additive genetic variance in the system. The component, σ_{AB}^2, is a part of the additive × additive epistatic variance. The value of V_m is now the simple sum of these three components. In summary the fundamental theorem for the present situation may be expressed as

$$\frac{d\bar{W}}{dt} = {\sigma_{10}}^2 + f{\sigma_{20}}^2 - 2\alpha_{AB}W_{11}rD \qquad [7]$$

where ${\sigma_{10}}^2$ and ${\sigma_{20}}^2$ stand for the additive genetic and additive × additive epistatic variances found in the system at the time, *t*, and *f* is the fraction of the additive × additive epistatic variance contained in V_m.

The inspection of the components in [6] shows that, as D approaches to zero,

$\sigma_A^2 \rightarrow \alpha_A^2/p(1-p)$

$\sigma_B^2 \rightarrow \alpha_B^2/q(1-q)$

$\sigma_{AB}^2 \rightarrow p(1-p)q(1-q)\alpha_{AB}^2$

At the limit, i.e., $D = 0$, these components become exactly the same as those given by COCKERHAM (1954). Under this condition the fraction, f, in [7] becomes $\frac{1}{2}$, and the rate of change in mean fitness becomes equal to $\sigma_{10}^2 + \frac{1}{2}\sigma_{20}^2$ at a given time.

KIMURA (1958) presented a fundamental theorem of natural selection which was completely general with respect to gene actions, mating systems and variable selection coefficients. When the generality in his formulation is reduced to that of the present study, KIMURA's theorem states that the rate of change in mean fitness is equal to the sum of additive genetic variance and another term due to joint effects of epistasis and linkage disequilibrium. It is not obvious whether the latter term is equal to $f\sigma_{20}^2 - 2\alpha_{AB}W_{11}rD$ in the present formulation. In the section titled "Application to Artificial Selection" in the same paper, KIMURA translated his theorem for dual epistasis of genes in linkage disequilibrium, and found that there existed some contributions from epistasis which were very similar in their expressions to the result of the present study. He did not, however, attempt to identify the epistatic contributions in terms of types of epistasis. In any event it is clear that there exists a variance contribution from epistasis to the rate of change in mean fitness, and that this contribution is due to the additive $\times$ additive epistasis.

DR. J. F. CROW has suggested that numerical demonstration of the theorem be given. For this purpose it is necessary to use genetic models with discrete generations. In such models the rates of change in gametic frequencies given in (1) are replaced by the differences between the corresponding gametic frequencies in two consecutive generations. Let Δx, Δy, Δz and Δu stand for these changes. Their expressions in terms of fitness values, gametic frequencies before selection, and recombination fractions are given by LEWONTIN and KOJIMA (1960), and they are equal to the right-hand sides of the equations in [1], each multiplied by $1/\overline{W}$. W's are now considered as Wrightian selective values rather than Malthusian parameters. Corresponding to $d\overline{W}/dt$ the difference between the means of two consecutive generations is

$$\Delta\overline{W} = \frac{1}{\overline{W}}\,[2V_m - 2\alpha_{AB}W_{11}rD]$$

or

$$\frac{1}{\overline{W}}\,[\sigma_{10}^2 + f\sigma_{20}^2 - 2\alpha_{AB}W_{11}rD] \qquad [8]$$

On the other hand, the mean fitness of the generation following selection, $\overline{W}'$, can be obtained by multiplying the fitness values in Table 2 with the new genotypic frequencies, $(x')^2 = (x + \Delta x)^2$, $(x'y') = (x + \Delta x)(y + \Delta y)$, - - -. The

difference, $(\overline{W}' - \overline{W})$, represents the actual change in mean fitness from one generation to the next. The values of $(\overline{W}' - \overline{W})$ and $\Delta\overline{W}$ do not agree exactly, since they are not instantaneous changes. The discrepancy is expressed by the quadratic form, $Q = gMg'$, where g and g' are the row and column vectors of Δx, Δy, Δz and Δu, and M is the matrix of fitness values in the form given in Table 2. Obviously, $\Delta\overline{W}$ is a linear function of the changes of the gametic frequencies, while Q is a homogeneous quadratic function of these changes.

TABLE 4

Fitness values for the numerical examples

	AA	*Aa*	*aa*
BB	1.0	0.7	0.4
Bb	0.6	0.5	0.4
bb	0.2	0.3	0.4

Matrix 1

	AA	*Aa*	*aa*
BB	0.7	0.8	0.9
Bb	0.7	0.6	0.5
bb	0.7	0.4	0.1

Matrix 2

	AA	*Aa*	*aa*
BB	1.1	0.7	0.1
Bb	0.6	0.5	0.2
bb	0.3	0.5	0.5

Matrix 3

Table 4 shows three sets of fitness values to be used in the numerical examples. To determine the sets of these fitness values in matrices 1, 2 and 3 the following conditions are considered.

(a) Dominance; Matrices 1 and 2 are arranged in such a way that there is no dominance among fitness values. The values in matrix 3 show dominance. In multiple-locus systems, no dominance at locus A may be defined as AA-$Aa = Aa$-aa for each of all possible combinations of genotypes at other loci. In matrices 1 and 2

$AA - Aa = Aa - aa$ for a given genotype at locus B and
$BB - Bb = Bb - bb$ for a given genotype at locus A.

(b) Epistasis; No epistasis may be defined by

$$W_{22} - W_{21} - W_{12} + W_{11} = e_{22} = 0$$
$$W_{21} - W_{20} - W_{11} + W_{10} = e_{21} = 0$$
$$W_{12} - W_{11} - W_{02} + W_{01} = e_{12} = 0$$
$$W_{11} - W_{10} - W_{01} + W_{00} = e_{11} = 0$$

according to COCKERHAM (1954). In all matrices given in Table 4, $e_{22} = e_{21} = e_{12} = e_{11}$ holds for each matrix. Matrix 1 has $e = 0.2$, Matrix 2 $e = -0.2$ and Matrix 3 $e = 0.3$.

The results of computations are presented in Table 5 (free recombination cases, $r = 0.5$) and in Table 6 (linkage cases, $r = 0.1$). The results generally indicate the following:

(a) $\Delta\overline{W}$ is a good indicator of $(\overline{W}' - \overline{W})$, the actual change in mean fitness. This holds true for various degrees of linkage disequilibrium and a wide range of

TABLE 5

Numerical demonstrations of the changes in mean fitness with two locus systems: free recombination between the two loci (r=0.5)

Matrix	1			2			3		
D*	0.00	+0.15	−0.15	0.00	+0.15	−0.15	0.00	+0.15	−0.15
$\overline{W}' - \overline{W}$	.0620	.0495	.0704	.0487	.1028	−.0026	.0740	.0423	.1020
$\Delta\overline{W}$	.0600	.0450	.0709	.0500	.1078	−.0027	.0728	.0371	.0970
Q	.0020	.0045	−.0005	−.0013	−.0050	.0001	.0012	.0052	.0050
$\sigma_{10}^2/\overline{W}$	.0500	.0660	.0295	.0417	.0617	.0197	.0504	.0631	.0317
$2V_m/\overline{W}$	.0600	.0718	.0368	.0500	.0670	.0245	.0728	.0753	.0498

* D= 0.00: x=0.25, y=0.25, z=0.25, u=0.25
D=+0.15: x=0.40, y=0.10, z=0.10, u=0.40
D=−0.15: x=0.10, y=0.40, z=0.40, u=0.10.

TABLE 6

Numerical demonstrations of the changes in mean fitness with two locus systems: linkage between the two loci (r=0.1)

Matrix	1		3	
D*	0.00	+0.15	0.00	+0.15
$\overline{W}' - \overline{W}$	.0620	.0710	.0740	.0727
$\Delta\overline{W}$	.0600	.0664	.0728	.0676
Q	.0020	.0046	.0012	.0051
$\sigma_{10}^2/\overline{W}$	.0500	.0660	.0504	.0631
$2V_m/\overline{W}$	.0600	.0718	.0728	.0753

* D=0.00: x=0.25, y=0.25, z=0.25, u=0.25
D=0.15: x=0.40, y=0.10, z=0.10, u=0.40.

recombination fraction. The existence of dominance does not seem to affect the agreement of $\Delta\overline{W}$ and $(\overline{W}' - \overline{W})$, particularly.

(b) With linkage disequilibrium and a large value of r, neither $\sigma_{10}^2/\overline{W}$ nor $2V_m/\overline{W}$ is a good indicator of $(\overline{W}' - \overline{W})$. When $D = 0$, however, $2V_m/\overline{W}$, i.e., $(\sigma_{10}^2 + \frac{1}{2}\sigma_{20}^2)/\overline{W}$ becomes equal to $\Delta\overline{W}$.

(c) With a tighter linkage, $2V_m/\overline{W}$ is a good indicator of $(\overline{W}' - \overline{W})$ usually. This is because the adjustment term, $-2\alpha_{AB}W_{11}rD$, becomes small in magnitude as r tends to zero. As r becomes smaller, the two-locus systems approach a one-locus system with multiple alleles. With $r = 0$, $2V_m$ becomes the additive genetic variance in a one-locus system with four alleles.

(d) One of the important findings is given by one of the examples with Matrix 2, $D = -0.15$ and $r = 0.5$ (Table 5). In this example $(\overline{W}' - \overline{W})$ itself is negative. Genetically speaking, this is caused by a large negative change in mean fitness associated with recombination. More precisely the change in $\overline{W}$ due to recombination in a system with linkage disequilibrium, $-2\alpha_{AB}W_{11}rD$, is negative, and the positive change in $\overline{W}$ from selection is not large enough to make the total change positive, as discussed in the previous section.

The consideration of these findings suggests that the fundamental theorem of natural selection may have to be stated in terms of a certain type of genetic covariance among relatives rather than the genetic variance in order to encompass general circumstances of epistasis and linkage. In fact, with $D = 0$ the rate of change in mean fitness is $\sigma_{10}^2 + \frac{1}{2}\sigma_{20}^2$, which is the covariance between a parent and all his potential offsprings before selection.

Genetic systems with more than two loci

In the preceding section the change of mean in a population with two segregating loci is examined in detail. With more than two loci the number of variables such as gene, gametic and zygotic frequencies increase very rapidly. In fact there are 2^n different gametes and 3^n different zygotes when the number of loci segregating in a population is n. Hence, it is straight forward but tiresome to spell out the expressions for change in each gametic frequency as was done for the case of $n = 2$. The only new consideration in deriving the change in gametic frequencies is to consider the contributions from the recombinations at triple, quadruple, . . . heterozygous loci, in addition to those at double heterozygous loci.

By the same procedure as the one used for $n = 2$, the rate of change in mean fitness over time is obtained as

$$\frac{d\bar{W}}{dt} = 2V_m - 2\Sigma W_H\left[\Sigma R\left\{\Sigma(x_i x_l - x_j x_k)(W_i - W_j - W_k + W_l)\right\}\right] \quad [9]$$

where V_m stands for the variance among the marginal means of all 2^n gametes, and W_H for the fitness value of various zygotes with two or more loci being heterozygous. x_i, x_j, x_k and x_l are the frequencies of gametes, i, j, k and l, respectively, and W_i, W_j, W_k and W_l are the corresponding gametic marginal means. The union of the gametes, i and l, and that of j and k produce the same heterozygote, (il) or (jk), of which the fitness value is W_H. It must be noted that there are 2^{n-2} different zygotes for each double heterozygote at a given pair of loci, 2^{n-3} for each triple heterozygote at a given set of three loci, and so forth. The summation on the left side of W_H in [9] is taken over all different double, triple, . . . heterozygotes.

R in [9] stands for the recombination fraction. When a double heterozygote is given, R represents a single recombination fraction between the two heterozygous loci. Given a triple heterozygote, say, *AaBbCc*, R represents one of the three recombination fractions (1) *between A* and *B* loci but *not between B* and *C* loci, (2) *between B* and *C* loci but *not between A* and *B* loci, or (3) *between A* and *B* loci and *simultaneously between B* and *C* loci. Generally the number of values R takes for a given h-fold heterozygote is $2^{(h-1)} - 1$. The summation on the left side of R is taken over all possible recombination fractions for a given heterozygote of which fitness is W_H.

The summation to the right of R is performed over different combinations of i, j, k and l for a given heterozygote with a given value of R in such a way that the recombinants from the zygote (il) are j and k, and those from (jk) are i and l.

As an illustration of the summation procedures, the case of $n = 3$ is considered in the following. Let 1, 2, 3, . . . and 8, stand for the gametes, *ABC*, *ABc*, *AbC*, *aBC*, *Abc*, *aBc*, *abC* and *abc*. The number of different zygotes for a given double heterozygote, say, *AaBb*, is 2 in this case; namely, *AaBbCC* and *AaBbcc*. The same is true for *AaCc* and *BbCc*, so that the total number of double heterozygote is 6. Now, given the zygote, say *AaBbCC*, the recombination which is effective can occur only between the loci *A* and *B*. The number of possible combinations of *i*, *j*, *k* and *l* gametes is only one, that is, ($i = 1$, $j = 3$, $k = 4$ and $l = 7$). Letting the recombination fraction for this case be r_{AB}, the contribution from the recombination in this double heterozygote to $d\overline{W}/dt$ becomes $-2W_H r_{AB}(x_1x_7 - x_3x_4) \times (W_1 - W_3 - W_4 + W_7)$ or $-2W_H r_{AB}(x_3x_4 - x_1x_7)(W_3 - W_1 - W_7 + W_4)$ where *H* stands for *AaBbCC*. There exist five similar contributions from the other five double heterozygotes.

Now there is only one triple heterozygote, *AaBbCc* in the system under consideration. As remarked before, *R* can take three values, $r_{AB}(1 - r_{BC})$, $(1 - r_{AB}) \times r_{BC}$ and $r_{AB}r_{BC}$, where *r* represents the recombination fraction between the subscripted loci. Given $R = r_{AB}(1 - r_{BC})$, one combination is ($i = 1$, $j = 4$, $k = 5$, $l = 8$) and the other is ($i = 2, j = 3, k = 6, l = 7$). Similarly for each of $(1 - r_{AB}) \times r_{BC}$ and $r_{AB}r_{BC}$, there are two combinations of *i*, *j*, *k* and *l*. The situation may be understood by the inspection of Diagram 1, in which all possible gametic combinations for the triple heterozygote are given in such a way that each combination is connected with all others by double headed arrows. Each arrow indicates that the combination of gametes connected with it are, reciprocally, recombinant and parental types, given the recombination fraction inserted on each arrow. When each combination of gametes is considered to be a zygote, then Diagram 1 represents the allocation of various types of the triple heterozygote and the recombi-

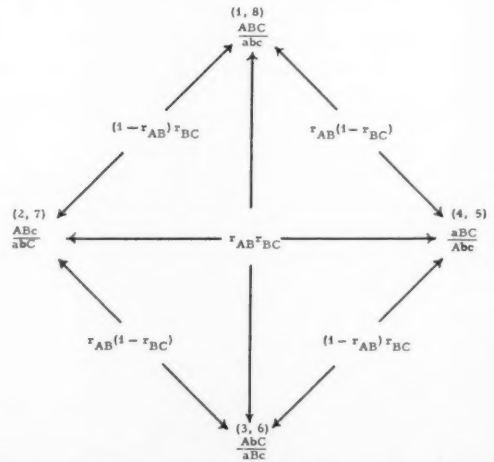


DIAGRAM 1.—Illustration of various types of triple heterozygotes and recombinational flows.

national flow among them. For example, x_1x_8 and x_2x_7 are the frequencies of types $\frac{ABC}{abc}$ and $\frac{ABc}{abC}$, respectively. If $x_1x_8 = x_2x_7$, there is no flow between them. If $x_1x_8 \neq x_2x_7$, then there is a flow from the type with high frequency to the one with low frequency, and the rate of the flow is $(1 - r_{AB})r_{BC}$. The same is true for all other types connected by the arrows.

Each of such flows is associated with a contribution to the rate of change in mean fitness. Thus, for the flow between $\frac{ABC}{abc}$ and $\frac{ABc}{abC}$ the size of the contribution is $-2r_{AB}(1 - r_{BC})(x_1x_8 - x_2x_7)W_H(W_1 - W_2 - W_7 + W_8)$, where W_H is the fitness value of the triple heterozygote and $(W_1 - W_2 - W_7 + W_8)$ represents the average differential advantage of gametes ABC and abc over gametes ABc and abC. The number of flows are six in total as shown in Diagram 1. Then the total contribution from the recombinations in the triple heterozygote to $d\overline{W}/dt$ is,

$$-2W_H\Big[r_{AB}(1-r_{BC})\Big\{(x_1x_8-x_4x_5)(W_1-W_4-W_5+W_8)+(x_2x_7-x_3x_6)(W_2-W_3-W_6+W_7)\Big\}+r_{BC}(1-r_{AB})\Big\{(x_1x_8-x_2x_7)(W_1-W_2-W_7+W_8)+(x_3x_6-x_4x_5)(W_3-W_4-W_5+W_6)\Big\}+r_{AB}r_{BC}\Big\{(x_1x_8-x_3x_6)(W_1-W_3-W_6+W_8)+(x_2x_7-x_4x_5)(W_2-W_4-W_5+W_7)\Big\}\Big]$$

where H stands for $AaBbCc$. In this fashion, one can construct the expression for the rate of change in $\overline{W}$ for any number of loci and obtain the general formula given in [9]. The terms such as $2W_HR(x_ix_l - x_jx_k)(W_i - W_j - W_k + W_l)$ may be called correction factors for linkage disequilibrium.

The marginal variance, V_m, in the theorem given by the formula [9] can be decomposed into the components due to the additive, additive × additive, additive × additive × additive, . . . gene effects. This variance consists of $2^n - 1$ comparisons, since there are 2^n marginal means. Among these, n comparisons are from the additive effects at individual loci, $\binom{n}{2}$ comparisons from the twofold additive effects, $\binom{n}{3}$ comparisons from the threefold additive effects, . . . and finally one comparison is from the n-fold additive effect.

As already indicated in the two-locus systems, the fractions of the additive type components of variance contained in the marginal variance depend upon the extent of linkage disequilibria represented by $(x_ix_l - x_jx_k)$ for various combinations of i, j, k and l. Generally, the expression for V_m may be written as

$$2V_m = \sigma_{10}^2 + f_{20}\sigma_{20}^2 + f_{30}\sigma_{30}^2 + --- = \sum_{i=1}^{n} f_{i0}\sigma_{i0}^2 \qquad [10]$$

where σ_{i0}^2 denotes the i-fold additive variance in the system and f_{i0} is the fraction of the i-fold additive variance contained in V_m. When the system approaches linkage equilibrium, f_{i0} tends to $(\frac{1}{2})^{i-1}$.

At the point of linkage equilibrium all the terms directly multiplied by $(x_ix_l - x_jx_k)$'s drop out, and the fundamental theorem of natural selection states

$$\frac{d\overline{W}}{dt} = 2V_m = \sum_{i=1}^{n} \left(\frac{1}{2}\right)^{i-1} \sigma_{i0}^2 \qquad [11]$$

When the population is not equilibrated with respect to linkage, the contributions due to the correction factors involving $(x_ix_l - x_jx_k)(W_i - W_j - W_k + W_l)$ to the rate of change in mean fitness are negative if $(x_ix_l - x_jx_k)$ and $(W_i - W_j - W_k + W_l)$ have the same sign, and otherwise they are positive. With many factors of this kind the rate of change in mean fitness can be much inflated or deflated from the rate expected from the size of the marginal variance. When the rate of change is deflated, it is possible that a large portion of $2V_m$ is cancelled by the contributions of this kind, so that the mean fitness will not change as much as one might predict from the magnitude of the marginal variance. As demonstrated for the case of $n = 2$, it may be possible with a large deflation that the total change in mean fitness becomes negative. With a large positive sum for the correction factors, on the other hand, the mean fitness will increase faster than the rate expected from the marginal variance. As an intermediate situation the positive and negative correction factors can cancel each other, and then the rate of change becomes approximately equal to the magnitude of $2V_m$. Generally, with a number of loci involved in determining fitness of individual organisms, the correction factors would tend to cancel and the limiting value of $d\overline{W}/dt$ would be $2V_m$.

Finally it should be noted that the influences of individual correction factors from high order heterozygotes such as quadruple ones, on the rate of change in $\overline{W}$ become progressively minute as the number of heterozygous loci increases. With five loci freely recombining, R for any double hetrozygote is $\frac{1}{2}$, while for the fivefold heterozygote $\frac{1}{16}$. As observed in some of the numerical examples given before, the influences of correction factors also become trivial as linkages among genes are tightened. When all individual recombination fractions are very small, the entire system may be treated as if the system consists of 2^n multiple alleles at a single locus. Under such a situation, the marginal variance, $\sum_{i=1}^{n} \left(\frac{1}{2}\right)^{i} \sigma_{i0}^2$, can be considered as one half of the additive genetic variance in the multiple allelic system.

SUMMARY

The change in mean fitness has been examined with respect to the effects of epistasis and linkage disequilibrium in gametic frequencies of fitness genes. With random mating it was found that the change in mean fitness was equal to twice the variance among the marginal means of all possible gametes plus a series of correction factors for linkage disequilibria. The variance among marginal means contains the additive genetic variance, and various fractions of all additive type

epistatic variance such as additive × additive, additive × additive × additive, . . . variances in such a way that the fractions become progressively small as the order of interaction increases. Individual correction factors may have positive or negative signs, so that some of the contributions from the correction factors may be cancelled in their summation with the result that the rate of change in mean fitness is approximately equal to the variance among marginal means.

As the degree of linkage disequilibrium is reduced, the change in mean fitness tends to $\sigma_{10}^2 + \frac{1}{-}\sigma_{20}^2 + \ldots (\frac{1}{-})^{i-1} \sigma_{i0}^2 + \ldots$ where σ_{i0}^2 stands for the i-fold additive epistatic variance. When the recombination fractions become very small, the magnitudes of the correction factors become trivial and the change in mean fitness can be equated to the variance among gametic marginal means which, in turn, may be redefined as the additive genetic variance with 2^n multiple alleles at a single locus.

It has been pointed out that the mean fitness of a population can decrease in spite of natural selection. This was effected by the high degree of breakdown of linkage disequilibrium among epistatic genes. Under this circumstance the original statement in the fundamental theorem of natural selection by FISHER must be modified to a certain extent.

ACKNOWLEDGMENT

The authors wish to thank DR. R. C. LEWONTIN for his criticisms and suggestions, particularly in the presentation of material on the two-locus system.

LITERATURE CITED

COCKERHAM, C. C., 1954 An extension of the concept of partitioning hereditary variance for analysis of covariances among relatives when epistasis is present. Genetics, **39**: 859–882.

CROW, J. F., and M. KIMURA, 1956 Some genetic problems in natural populations. Proc. Third Berkeley Symp. Math. Stat. Prob., **4**: 1–22.

FISHER, R. A., 1930 *The Genetical Theory of Natural Selection.* Clarendon Press. Oxford, England.

KEMPTHORNE, O., 1954 The correlation between relatives in a random mating population. Proc. Roy. Soc. London, B **143**: 103–113.

KIMURA, M., 1956 A model of a genetic system which leads to closer linkage by natural selection. Evolution **10**: 278–287.

1958 On the change of population fitness by natural selection. Heredity **22**: 145–167.

KING, J. C., 1955 Evidence for the integration of the gene pool from studies of DDT resistance in Drosophila. Cold Spring Harbor Symposia Quant. Biol. **20**: 311–317.

LEWONTIN, R. C., 1958 A general method for investigating the equilibrium of gene frequency in a population. Genetics **43**: 421–433.

LEWONTIN, R. C., and K. KOJIMA, 1960 The evolutionary dynamics of complex polymorphisms. Evolution **14**: 458–472.

LI, C. C., 1955 The stability of an equilibrium and the average fitness of a population. Am. Naturalist **89**: 281–295.

VETUKHIV, M., 1953 Viability of hybrids between local populations of *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. U.S. **39**: 30–34.

WRIGHT, S., 1949 Adaptation and selection. Pp. 365–389, *Genetics, Paleontology*, and *Evolution*. Princeton Press. Princeton, N.J.

1955 Classification of the factors of evolution. Cold Spring Harbor Symposia Quant. Biol. **20**: 16–24.

THE S SYSTEM OF BOVINE BLOOD GROUPS

CLYDE STORMONT, W. J. MILLER AND YOSHIKO SUZUKI

The Serology Laboratory, Department of Microbiology,
University of California, Davis, California

Received December 12, 1960

THIS report is concerned with the bovine blood factors named S, U_1, U_2 and H′ (FERGUSON, STORMONT and IRWIN 1942; STORMONT 1950) which came to be assigned to two separate genetic systems of cattle blood (see STORMONT 1954; IRWIN 1955, 1956; also see discussions by NEIMANN-SORENSEN 1958; RENDEL 1958). As will be shown in this report, those four blood factors actually belong to the same genetic system, a system referred to hereafter as the S system of bovine blood groups.

MATERIALS AND METHODS

The blood-typing reagents used in this study are designated S, U_1, U_2, S_2, H′ and U′. The S, U_1 and U_2 reagents were known replicas of S, U_1 and U_2 reagents used by STORMONT (1950) in the first analysis of serological relationships involving blood factors S, U_1 and U_2. In that report, a blood-typing reagent was described which reacted with the bloods of all cattle of phenotypes S, U_2, SU_1 and SU_2 but not with bloods of cattle of phenotypes U_1 and − (i.e., SU-negative bloods) thereby establishing a close serological relationship between blood factors S and U. Because of the perfect correlation of the reactions of that reagent with the presence of either or both blood factors S and U_2, STORMONT named it (SU_2), in which symbolism the parentheses were intended to indicate that the antibodies were not fractionable by absorption. In this report the (SU_2) reagent and its equivalent blood factor are designated by means of the much simpler symbol S_2.

The S_2 reagent used in the present study was prepared from an aliquot of the 26Z (6g) isoimmune serum (see STORMONT 1950), which was the original source-antiserum for (SU_2) reagent. Because of the small supply of 26Z (6g) antiserum, the S_2 reagent was used very sparingly in the present study. When U′ reagents (STONE and MILLER 1961) were reproduced in this laboratory, the use of S_2 reagent was discontinued since U′, as demonstrated elsewhere in this report, serves as an effective substitute for S_2 reagent in distinguishing phenotypes in the S system.

The H′ reagents of this study were known replicas of the first H′ reagent obtained from bovine isoimmune serum (STORMONT 1947). Actually, all H′ reagents now in use in the various cattle blood-typing laboratories are replicas of that H′ reagent. We emphasize this point of reference since it is now known, as discussed elsewhere in this report, that contemporary H′ reagents are not of the same specificity as the original H′ reagent described by FERGUSON *et al.* (1942).

One of the H′ reagents used in the present study, namely, an H′ reagent obtained from a rabbit antiserum coded RC35, served effectively as a dosage reagent.

The blood samples used in the family studies were essentially the same as those used in the report on the A system of bovine blood groups (STORMONT 1961) except that those samples were supplemented by addition of samples from the families of eight Holstein-Friesian bulls of phenotype SU_1H' (see Table 2) owned by various artificial breeding organizations in United States. In all, there were 95 sire-families with a total of 2,723 offspring for an average of approximately 28.7 offspring per family.

Lytic tests, in which pooled, fresh-frozen serum from selected rabbits was used as complement, were performed as described in a number of the reports already cited.

RESULTS

The nine phenotypes defined to date in tests of representatives of various breeds of *Bos taurus* with the reagents S, U_1, U_2, S_2, H′ and U′ are shown in Table 1.

Attention is called first to the fact that the phenotype designations U_2, SH′, U_1H', U_2H', SU_1H', SU_2H' and U_1U_2H' are actually shorthand designations for the phenotypes U_2S_2U', SS_2H', U_1U_2H', $U_2S_2H'U'$, $SU_1U_2S_2H'$, $SU_2S_2H'U'$ and $U_1U_2S_2H'U'$. As may be noted by inspecting the reactions shown in Table 1, the main utility of reagents S_2 and U′ is that of distinguishing phenotype U_1H' from U_1U_2H' (cf. discussion in reports by STORMONT 1950; STONE and MILLER 1961). In that capacity, the two reagents are equally effective. Consequently, once U′ reagents were developed in this laboratory we were able to discontinue use of the S_2 reagent.

TABLE 1

Nine phenotypes in the S system of Bos taurus *showing the serological reactions which define them*

Phenotypes	Reactions with the reagents* S	U_1	U_2	S_2	H′	U′	Diploid combinations of the five postulated phenogroups†
—	0	0	0	0	0	0	—/—
U_2	0	0	+	+	0	+	U_2/—, U_2/U_2
H′	0	0	0	0	+	0	H′/—, H′/H′
SH′	+	0	0	+	+	0	SH′/—, SH′/H′, SH′/SH′
U_1H'	0	+	+	0	+	0	U_1H'/—, U_1H'/H', U_1H'/U_1H'
U_2H'	0	0	+	+	+	+	U_2/H'
SU_1H'	+	+	+	+	+	0	SH'/U_1H'
SU_2H'	+	0	+	+	+	+	SH'/U_2
U_1U_2H'	0	+	+	+	+	+	U_1H'/U_2

* Reagents of specificity U_2 were originally designated as U reagents (FERGUSON *et al.* 1942; STORMONT 1950). The S_2 reagent of this study was formerly designated as the (SU_2) reagent (STORMONT 1950).

† See discussion in text.

TABLE 2

Summary of the results of progeny testing 95 bulls with respect to their genotypes at the S locus

Phenotypes	Genotypes	Number of bulls by breeds* showing number of offspring within parentheses				
		AA	G	H	HF	J
1. —	ss	6 (302)		3 (54)	8 (290)	5 (138)
2. H′	$s^{H'}s$	4 (192)		4 (129)	12 (293)	3 (17)
3. H′	$s^{H'}s^{H'}$	1 (5)		1 (7)	4 (208)	5 (180)
4. SH′	$s^{SH'}s$	1 (29)	5 (186)		4 (51)	4 (122)
5. SH′	$s^{SH'}s^{H'}$		2 (25)		2 (25)	3 (50)
6. SH′	$s^{SH'}s^{SH'}$		1 (154)			5 (115)
7. U_1H'	$s^{U_1H'}s$				1 (11)	
8. U_1H'	$s^{U_1H'}s^{H'}$				2 (92)	
9. U_2H'	$s^{U_2s}H'$				1 (16)	
10. SU_1H'	$s^{SH'}s^{U_1H'}$				8 (32)	

* AA (Aberdeen-Angus), G (Guernsey), H (Hereford), HF (Holstein-Friesian), J (Jersey).

Subtyping relationships of blood factors S, U_1, U_2, S_2, H′ and U′: Asymmetries in the occurrence of combinations of reactions with blood-typing reagents are spoken of as subtyping relationships. A number of subtyping relationships involving both linear and nonlinear patterns of cross reactions have already been described in the cattle studies (STORMONT 1950, 1955) and many more are yet to be described (MILLER, SUZUKI and STORMONT 1961).

Those subtyping relationships involving linear patterns of cross reactions are generally, although not invariably, indicated by special symbolic designations, as C_1 and C_2, O_1, O_2 and O_3, T_1 and T_2, U_1 and U_2, . . . etc. For example, the bovine blood factors named C and E by FERGUSON (1941) were observed in only three of four possible phenotypic combinations. The observed combinations were C and E together, C alone and no-CE. In keeping with subtyping designations, it was suggested (STORMONT 1950) that the combination C and E together be indicated by the symbol C_1 and C alone by the symbol C_2. Likewise, the bovine blood factors B and K were observed in only three of four phenotypic combinations, namely, BK, B alone and no-BK, and the same was true of G and K but B and G were observed in all four possible combinations, as evidenced by examining the five recognized phenotypic combinations of blood factors B, G and K, namely, BGK, BG, B, G and no-BGK (STORMONT 1950). Consequently in that and similar situations in which both linear and nonlinear patterns of cross reactions were involved, subtyping symbolism was avoided. Much discussion has been made about the immunogenetic implications of the BGK relationship but that relationship is by no means unique as evidenced by the relationships now described.

Attention is called first to the reactions of reagents S, S_2 and H′ in Table 1. The observed phenotypic combinations of those reactions are SS_2H', S_2H', S_2, H′ and no-SS_2H'. It is apparent that this pattern of reactions is a repetition of the subtyping relationships exhibited by blood factors B, G and K, with blood factor S playing the role of K. The present significance of the observed relationships exhibited by blood factors S, S_2 and H′ was that they provided the first line of

evidence that blood factor H′ must belong to the same blood-group system as blood factors S and S_2.

Likewise, we note from the data presented in Table 1 that the phenotypic combinations of blood factors U_1, U_2 and H′ provide another BGK-like relationship in which blood factor U_1 plays the role of K. The present significance of the observed subtyping relationships involving U_1, U_2 and H′ was that those relationships provided another independent line of evidence relating blood factor H′ to the S system.

Finally, attention is called to still a third BGK-like relationship involving the blood factors S_2, U_2 and U′, in which U′ plays the role of K.

The subtyping interrelationships of blood factors S, U_1, U_2, S_2, H′ and U′ provide the strongest kind of evidence that those blood factors all belong to the same system of blood groups.

Phenogroups in the S system: The inability to resolve subtyping relationships on the basis of the concept of unit antigens alleged to be controlled by single genes (see STORMONT 1950; STORMONT, OWEN and IRWIN 1951) led to the use of the term "blood factor" or "serological factor" (LANDSTEINER 1945) as a replacement for such terms as "unit antigen", "antigenic substance", "antigenic character" and so on. It also led to the concept "phenogroup" although the term itself was not introduced until over a decade later (STORMONT 1955). The concept phenogroup, which is equivalent in all respects to the concept "agglutinogen" (WIENER and WEXLER 1952), holds that subtyping relationships are merely the reflections of reciprocal and nonreciprocal cross reactions of a series of immunochemically related structures on the surfaces of red cells. These hypothetical structures are referred to as phenogroups in the cattle and sheep studies (RASMUSEN 1960).

The question arises, "What is the minimum number of phenogroups that need be postulated to account for the patterns of reactions shown in Table 1?" The answer is five, as now demonstrated.

The five phenogroups postulated to account for the cross reactions with S, U_1, U_2, S_2, H′ and U′ antibodies are designated −, U_2, H′, SH′ and U_1H′.

The postulated phenogroup "−" is characterized by its inability to react with any of the antibodies in the reagents S, U_1, U_2, S_2, H′ and U′. In the diploid combination −/−, that phenogroup would account for phenotype "−" in Table 1.

The postulated phenogroup U_2 is characterized by reactions with U_2, S_2 and U′ antibodies. In the diploid combinations U_2/− and U_2/U_2, phenogroup U_2 would account for the phenotype U_2 in Table 1.

The postulated phenogroup H′ is characterized by its reactions with H′ antibodies. In the diploid combinations H′/− and H′/H′, it would account for phenotype H′ in Table 1.

The postulated phenogroup SH′ is characterized by reactions with S, S_2 and H′ antibodies. In the diploid combinations SH′/−, SH′/H′ and SH′/SH′, it would account for phenotype SH′ in Table 1.

The postulated phenogroup U_1H′ is characterized by reactions with U_1, U_2 and

H′ antibodies. In the diploid combinations $U_1H'/-$, U_1H'/H' and U_1H'/U_1H', it would account for phenotype U_1H' in Table 1.

Finally, the four remaining diploid combinations U_2/H', SH'/U_1H', SH'/U_2 and U_1H'/U_2 would account for, respectively, the phenotypes U_2H', SU_1H', SU_2H' and U_1U_2H' in Table 1.

It is implied, although it is not a necessary part of the concept phenogroup, that the immunochemically related structures postulated to account for the cross reactions of such antibodies as S, U_1, U_2, S_2, H′ and U′ are controlled by genes that are closely related in evolutionary descent. Such genes are generally referred to in genetics as alleles. It is now shown that the phenogroups −, U_2, H′, SH′ and U_1H' do segregate in inheritance as if controlled by allelic genes.

Progeny tests: Six of the nine phenotypes shown in Table 1 were represented in the 95 bulls which headed the sire-families of this study. Those were the phenotypes −, H′, SH′, U_1H', U_2H' and SU_1H'. Corresponding to those six phenotypes, ten genotypes were proved in the progeny tests of the bulls as summarized in Table 2. Represented in the ten genotypes were the five alleles s, s^{U_2}, $s^{H'}$, $s^{SH'}$ and $s^{U_1H'}$ corresponding, respectively, to the five postulated phenogroups −, U_2, H′, SH′ and U_1H'.

Details of the evidence, excepting the progeny tests involving the six bulls (line 6 of Table 2) which proved to be of genotype $s^{SH'}s^{SH'}$, are presented in Tables 3–11. All except one of the 269 offspring of the six bulls of genotype $s^{SH'}s^{SH'}$ were of phenotype SH′. The one was of phenotype SU_1H' (presumably genotype $s^{SH'}s^{U_1H'}$), and she resulted from a mating to a dam of that same phenotype.

Table 3 is a summary of the matings of the 22 bulls of phenogroup-formula $-/-$. The results in that table are consistent with the proposal that all animals of phenotype "−" are of genotype *ss*.

Table 4 is a summary of the matings of the 23 bulls (line 2 of Table 2) which bred as if of genotype $s^{H'}s$. The observed ratio of 138:122 in matings to "negative" dams is consistent with the proposal that those bulls were of genotype $s^{H'}s$. Furthermore, their red cells exhibited the "weak" type of reactions in tests with dosage H′ reagent.

TABLE 3

Summary of the matings of 22 bulls of phenogroup-formula $-/-$ and genotype ss *(see line 1 of Table 2)*

Matings Males × Females	Number of offspring of phenotypes				
	−	U_2	H′	SH′	U_1H'
− × −	202	0	0	0	0
U_2	5	3	0	0	0
H′	160	0	303	0	0
SH′	21	0	22	54	0
U_1H'	1	0	1	0	6
U_2H'	0	5	1	0	0

TABLE 4

Segregation of phenogroups H′ and — as alternatives in 631 progeny of 23 bulls of phenogroup-formula H′/— and genotype $s^{H'}$*s (see line 2 of Table 2)*

Matings Males × Females	—	Number of offspring of phenotypes U_2	H′	SH′	U_1H'	U_2H'
H′ × —	138	0	122	0	0	0
U_2	0	3	5	0	0	2
H′	73	0	240	0	0	0
SH′	6	0	9	26	0	0
U_1H'	0	0	1	0	2	0
U_2H'	0	0	2	0	0	2

TABLE 5

Summary of the matings of 11 bulls of phenotype-formula H′/H′ and genotype $s^{H'}s^{H'}$ *(see line 3 of Table 2)*

Matings Males × Females	Number of offspring of phenotypes H′	SH′	U_1H'	U_2H'
H′ × —	92	0	0	0
U_2	0	0	0	2
H′	189	0	0	0
SH′	51	62	0	0
U_1H'	1	0	3	0

Table 5 is a summary of the matings of the 11 bulls (line 3 of Table 2) which bred as if of genotype $s^{H'}s^{H'}$. Their red cells exhibited the "strong" type of reactions with dosage H′ reagents.

Table 6 shows the segregation of phenogroups SH′ and — in the progeny of 14 bulls (line 4 of Table 2) which bred as if of genotype $s^{SH'}s$, and whose red cells exhibited the weak reaction with dosage H′ reagent. In matings to negative dams the expected ratio of SH′ to — was 1:1 and the observed ratio was 28:23.

Table 7 shows the segregation of phenogroups SH′ and H′ as alternatives in the progeny of the seven bulls (line 5 of Table 2) which bred as if of genotype $s^{SH'}s^{H'}$ and whose red cells exhibited the strong reaction with dosage H′ reagent.

TABLE 6

Segregation of phenogroups SH′ and — as alternatives in 388 progeny of 14 bulls of phenogroup-formula SH′/— and genotype $s^{SH'}$*s (see line 4 of Table 2)*

Matings Males × Females	—	Number of offspring of phenotypes H′	SH′	U_1H'	SU_2H'
SH′ × —	28	0	23	0	0
U_2	2	0	0	0	2
H′	9	50	52	0	0
SH′	15	17	185	0	0
SU_1H'	0	0	4	1	0

The test matings to negative dams and dams of phenotype H′ were expected to produce a 1:1 ratio of H′ to SH′. The observed ratio was 6+18:5+27.

Table 8 shows the segregation of phenogroups U_1H' and — as alternatives in the 11 progeny of the one bull (line 7 of Table 2) which bred as if of genotype $s^{U_1H'}s$ and whose red cells exhibited the weak reaction with dosage H′ reagent.

Table 9 shows the segregation of phenogroups U_1H' and H′ as alternatives in the offspring of the two bulls (line 8 of Table 2) which bred as if of genotype $s^{U_1H'}s^{H'}$ and whose red cells exhibited the strong reaction with dosage H′ reagent. A 1:1 ratio was expected in matings of those bulls to negative cows and cows of phenotype H′. The observed ratio was 16+29:12+32.

Table 10 shows the segregation of phenogroups U_2 and H′ as alternatives in the 16 progeny of the one bull (line 9 of Table 2) which bred as if of genotype $s^{U_2}s^{H'}$ and whose red cells showed weak reaction with dosage H′ reagent. In

TABLE 7

Segregation of phenogroups SH′ and H′ as alternatives in 100 offspring of seven bulls of phenogroup-formula SH′/H′ and of genotype $s^{SH'}s^{H'}$ *(see line 5 of Table 2)*

Matings Males × Females	Number of offspring of phenotypes H′	SH′
SH′ × —	6	5
H′	18	27
SH′	6	37
U_1H'	0	1

TABLE 8

Segregation of the phenogroups U_1H' *and — as alternatives in 11 offspring of a bull of phenogroup-formula* $U_1H'/-$ *and of genotype* $s^{U_1H'}s$ *(see line 7 of Table 2)*

Matings Males × Females	Number of offspring of phenotypes —	H′	U_1H'
U_1H' × —	2	0	1
H′	0	2	5
U_1H'	1	0	0

TABLE 9

Segregation of phenogroups U_1H' *and H′ as alternatives in 92 progeny of two bulls of phenogroup-formula* U_1H'/H' *and genotype* $s^{U_1H'}s^{H'}$ *(see line 8 of Table 2)*

Matings Males × Females	Number of offspring of phenotypes H′	SH′	U_1H'	SU_1H'
U_1H' × —	16	0	12	0
H′	29	0	32	0
SH′	0	1	0	1
U_1H'	0	0	1	0

$U_2H' \times -$ matings we should expect a 1:1 ratio of U_2 to H′. By combining the results in line 1 of Table 10 with those in the last line of Table 3, the observed ratio was 1+5:2+1.

Table 11 shows the segregation of phenogroups SH′ and U_1H' as alternatives in the progeny of the eight bulls (line 10 of Table 2) which bred as if of genotype $s^{SH'}s^{U_1H'}$. A 1:1 ratio of SH′ to U_1H' is expected in $SU_1H' \times -$ matings and also in $SU_1H' \times H'$ matings. The observed ratio was 1+10:1+8.

Attention is called to the fact that there were no matings involving the rare phenotypes SU_2H' and U_1U_2H'. There were, however, four offspring of phenotype SU_2H' (Tables 6 and 10) and one of phenotype U_1U_2H' (Table 10).

Estimates of the frequencies of the alleles which control the phenogroups −, U_2, H′, SH′ and U_1H' in Guernsey, Holstein-Friesian and Jersey cattle in United States are given in another report (STORMONT 1958). Those estimates were based on very small samples. It is our intention to submit elsewhere gene-frequency data based upon considerably larger samples of various populations of *Bos taurus*.

TABLE 10

Segregation of phenogroups U_2 and H′ as alternatives in 16 offspring of a bull of phenogroup-formula U_2/H' and genotype $s^{U_2}s^{H'}$ (see line 9 of Table 2)

Matings Males × Females	U_2	H′	Number of offspring of phenotypes SH′	U_2H'	SU_2H'	U_1U_2H'
$U_2H' \times -$	1	2	0	0	0	0
H′	1	4	0	2	0	0
SH′	1	0	2	0	2	0
U_1H'	0	0	0	0	0	1

TABLE 11

Segregation of phenogroups SH′ and U_1H' as alternatives in 32 offspring of eight bulls of phenogroup-formula SH'/U_1H' and genotype $s^{SH'}s^{U_1H'}$ (see line 10 of Table 2)

Matings Males × Females	Number of offspring of phenotypes SH′	U_1H'	SU_1H'
$SU_1H' \times -$	1	1	0
H′	10	8	0
SH′	7	2	1
U_1H'	1	0	1

DISCUSSION

The foregoing analysis of the S system of bovine blood groups was presented in a series of four steps. First we considered the nine S-system phenotypes in *Bos taurus* and the reactions which defined those phenotypes. The second step was concerned with a description of the subtyping relationships exhibited by the blood factors S, U_1, U_2, S_2, H′ and U′. The third step was concerned with an

analysis of the number of phenogroups in *Bos taurus* that need be postulated to account for the observed phenotypes. The fourth and final step was an analysis of family data which, in retrospect, simply provided genetic proof for the existence of the five postulated phenogroups. Thus, serological relationships alone can serve in lieu of genetic tests in the analysis of blood-group systems. Such an approach may have application in marine population research where genetic tests are not possible. The postulated phenogroups can then be tested by gene-frequency analysis.

The results of this analysis of S-system phenogroups may be contrasted with recent studies (STORMONT, MILLER and SUZUKI 1961) of S-system phenogroups in American buffalo (*Bison bison* or *Bos americanus*). Only two S-system phenogroups were distinguished in the bison studies. One of those two phenogroups was equivalent to the "—" phenogroup of *Bos taurus*. The other, designated S_b, was characterized by reactions with S reagent and by weak, partial cross reactions with one of two U_1+H′ reagents. (S_2 reagent was not used in those studies.) We were able to show that S-positive bison bloods will readily absorb S antibodies in S+H′ reagents without affecting the H′ antibodies. Whether a phenogroup equivalent in specificity to S_b exists in *Bos taurus* is an open question. In view of the fact that most bison phenogroups have homologues or near-homologues in *taurus*, it is suggestive that such a phenogroup may one day be found in *taurus*.

It was stated elsewhere in this report that contemporary H′ reagents cannot be of the same specificity as the original H′ described by FERGUSON *et al.* (1942). Some of the evidence for that statement was obtained in examining the S and H′ reactions shown in Table 1 of the report by FERGUSON *et al.* (1942) in which table it is seen that blood factor S occurred with and without H′, which is not in keeping with the results reported here. The original source-antiserum for H′ antibodies was exhausted very early in those studies and, as may be seen in the report by STORMONT *et al.* (1951), H′ was not among the 38 blood factors examined in the analyses of the blood group systems B, C, F-V and Z. Actually, those studies were conducted largely over the years 1941 to 1944. We cannot, therefore, be certain just when the transition in H′ reagents took place. But the important point, it seems, is to note here that the blood factor now called H′ bears no known relationship to the original H′ of FERGUSON *et al.* (1942).

In the studies of the B, C, F-V and Z systems of cattle (STORMONT, OWEN and IRWIN 1951; STORMONT 1952) the blood factors left unassigned were A, H, J, S, U_1, U_2 and Z′. J, of course, represented a special case of a blood factor which is only a secondary attribute of the red cells (STORMONT 1949). Studies of the genetics of the J property were reported by STONE and IRWIN (1954) and by SPRAGUE (1958). The basis for the assignment of A, H and Z′ to the A system, along with the more recently described blood factor D, is given in another report (STORMONT 1961). S, U_1 and U_2, along with contemporary H′, were shown in this report to be members of the S system, thereby completing the analysis of the genetic positions of the 38 bovine blood factors examined by STORMONT *et al.* (1951).

Tests for independence of the seven loci (A, B, C, F-V, J, S and Z) implicated in the control of those 38 blood factors along with evidence for two additional systems are to be presented in a following report.

SUMMARY

On the basis of an analysis of the cross reactions of the bovine blood-typing reagents named S, S_2 (formerly designated as (SU_2) reagent), U_1, U_2, H′ and U′, five phenogroups designated "—", U_2, H′, SH′ and U_1H' are postulated to account for the equivalent blood factors. Genetic evidence is then offered in proof of the existence of the five phenogroups which segregate as if controlled by allelic genes. The alleles are designated, respectively, as s, s^{U_2}, $s^{H'}$, $s^{SH'}$ and $s^{U_1H'}$ in which symbolism the phenogroups are written as superscripts.

Two subtyping relationships, one involving the trio of blood factors SS_2H' and the other involving the trio U_1U_2H' provided the first lines of evidence that blood factor H′, contrary to previous opinion, is a member of the S system. It is pointed out that those subtyping relationships parallel the often discussed BGK relationship.

In the course of study it became evident that contemporary H′ reagents bear no known relationship to the original H′ reagent described in 1942.

ACKNOWLEDGMENT

We wish to acknowledge the assistance of MR. B. G. MORRIS and DR. B. A. RASMUSEN in the collection of much of the data. We also wish to acknowledge the collaboration of MESSRS. J. C. HERSHEY, R. GAMBONINI, M. J. FOLEY, E. E. GREENOUGH, O. C. BORLAND, R. C. LABEN, P. W. GREGORY, S. W. MEAD and W. C. ROLLINS in the collection of blood samples and in the provision of family pedigrees.

LITERATURE CITED

FERGUSON, L. C., 1941 Heritable antigens in the erythrocytes of cattle. J. Immunol. **40**: 213–242.

FERGUSON, L. C., C. STORMONT, and M. R. IRWIN, 1942 On additional antigens in the erythrocytes of cattle. J. Immunol. **44**: 147–164.

IRWIN, M. R., 1955 III. Immunogenetics. Pp. 55–71. *Biological Specificity and Growth.* Edited by E. G. BUTLER. Princeton University Press. Princeton, N.J.

1956 Blood grouping and its utilization in animal breeding. 7th Intern. Congr. Anim. Husbandry. Madrid **2**: 7–41.

LANDSTEINER, K., 1945 *The Specificity of Serological Reactions.* Harvard University Press. Cambridge, Massachusetts.

MILLER, W. J., Y. SUZUKI, and C. STORMONT, 1961 Subtypes in cattle blood typing. (In preparation.)

NEIMANN-SORENSEN, A., 1958 Blood groups of cattle. Thesis. A/S Carl Fr. Mortensen, Copenhagen.

RASMUSEN, B. A., 1960 Blood groups in sheep. II. The B system. Genetics **45**: 1405–1417.

RENDEL, J., 1958 Studies of cattle blood groups. I. Production of cattle iso-immune sera and the inheritance of 4 antigenic factors. Acta. Agr. Scand. **8**: 40–61.

SPRAGUE, L. M., 1958 On the recognition and inheritance of the soluble blood group property "Oc" of cattle. Genetics. **43**: 906–912.

STONE, W. H., and M. R. IRWIN, 1954 The J substance of cattle. I. Developmental and immunogenetic studies. J. Immunol. **73**: 397–406.

STONE, W. H., and W. J. MILLER, 1961 Naturally occurring isoantibodies of the S blood group system in cattle. J. Immunol. **86**: 165–169.

STORMONT, C., 1947 Unpublished observations. Records Agr. Exp. Stat. University of Wisconsin.

1949 Acquisition of the J substance by the bovine erythrocyte. Proc. Natl. Acad. Sci. U.S. **35**: 232–237.

1950 Additional gene-controlled antigenic factors in the bovine erythrocyte. Genetics **35**: 76–94.

1952 The F-V and Z systems of bovine blood groups. Genetics **37**: 39–48.

1954 Erythrocyte mosaicism in a heifer recorded as single-born. J. Animal Sci. **13**: 94–98.

1955 Linked genes, pseudoalleles and blood groups. Am. Naturalist **89**: 105–116.

1958 On the applications of blood groups in animal breeding. Proc. 10th Intern. Congr. Genet. **1**: 206–224.

1961 The A system of bovine blood groups. Genetics **46**: (In press).

STORMONT, C., R. D. OWEN, and M. R. IRWIN, 1951 The B and C systems of bovine blood groups. Genetics **36**: 134–161.

STORMONT, C., W. J. MILLER and Y. SUZUKI, 1961 Blood groups and the taxonomic status of American buffalo and domestic cattle. Evolution. (In press.)

WIENER, A. S., and I. B. WEXLER, 1952 The mosaic structure of red cell agglutinogens. Bacteriol. Rev. **16**: 69–87.

RELATIVE FITNESS OF GENETICALLY OPEN AND CLOSED EXPERIMENTAL POPULATIONS OF DROSOPHILA ROBUSTA

HAMPTON L. CARSON

Department of Zoology, Washington University, St. Louis, Missouri

Received December 16, 1960

WHEN a genetically variable gene pool faces a new environmental challenge, microevolutionary change may be expected to occur. To understand this change, it would be ideal to know the details of the structure and integration of the gene pool both before and after the change. Although such conditions can hardly be met in any study of natural populations, an approach, at least, can be made in laboratory experiments.

In a number of species of Drosophila, genetic control is now such that the artificial construction of various kinds of gene pools presents no particular difficulty. The classical methods for the experimental study of gene pools in the laboratory (L'HÉRITIER, NEEFS and TEISSIER 1937; WRIGHT and DOBZHANSKY 1946; REED and REED 1948; BUZZATI-TRAVERSO 1955) provide an opportunity for the application of stringent natural selection and afford some control of population size. These experimental designs, however, provide no means for comparing the over-all biological performance of one gene pool with another when both are maintained under similar environmental conditions. By providing exact amounts of food and space and exact change intervals to various genetically different experimental populations of *D. melanogaster*, CARSON (1958c; 1961), BERT (1960) and SMATHERS (1961) developed methods for making weekly population size measurements under the arbitrarily imposed conditions. CARSON (1961) has proposed that population size, measured for comparable gene pools under these conditions, may be used as a measure of the performance of the population, or, in short, its relative population fitness. The gene pool which maintains the larger size is judged the relatively more fit.

The present paper concerns itself with the extension of this method to laboratory populations of *Drosophila robusta*. This species is a common native faunal element of the deciduous forest of the eastern United States, having a large number of different gene arrangements which generally show clinal distributions and a strong tendency towards marginal homozygosity (CARSON 1955, 1958a, 1959).

Data are presented in this paper on laboratory populations derived from various geographic sources, both marginal and central. Some of the populations studied are genetically "closed" throughout the course of the experiment. Others have been kept genetically "open" by maintaining a continual inflow of genes; still others have been subjected to mass hybridization. The performance of these various populations and their controls has been compared, and the fate of chromosome polymorphism carried by them has been followed.

MATERIAL AND METHODS

Samples of *Drosophila robusta* were obtained from natural populations according to the usual methods of fruit baiting. This procedure is now known to provide a truly representative sample of the adult wild feeding population of this species (see CARSON 1958b, p. 28). Immediately upon collection of the wild samples from the natural population, males and females were separated and each female placed individually or with a single wild male into a separate culture vial. Salivary gland smears were made from her offspring using acetolactoorcein. In some cases wild females can be shown to have previously mated to two or possibly more different wild males in nature. One has no assurance, therefore, that a culture established from a single wild-caught female is descended from a single pair of wild flies. A newly caught female, however, may be rendered free of sperm from wild males by placing her individually in a culture tube and changing to a fresh vial often (every one or two days) until the eggs laid by the female no longer hatch. The female concerned may then be mated to a single wild male caught at the same time and place. This procedure has been used in those cases where it is desirable to know the precise number of wild homologs entering into a laboratory strain by way of the founders (see also CARSON 1958a).

A method for establishing and maintaining an experimental population in vials has been described in detail by CARSON (1961). With only minor modifications, this method was employed in the present work. Basically, the adult flies are maintained in a chamber made of an ordinary 25 × 95 mm shell vial to which is taped a cellulose acetate tube of the same size ("supervial"). The latter contains a platform of blotting paper and is plugged with cotton at the free end. The shell vial, which contains the food, is replaced at exact intervals by opening the chamber, holding the adult flies in the supervial, removing the old vial and taping a new one in position. In most of the populations to be reported on in this paper, four changes a week were made. Each food vial contained 9.5 cc of a specially prepared uniform corn meal-Karo-agar medium, to which has been added ten mg of dry Fleishmann's yeast moistened with one drop of distilled water.

Each time the population chamber was opened, flies which had emerged in vials which were previously in contact with the population were added to the adult population. Under these conditions, the size of the population eventually comes to equilibrium with the space and food available. In the experiments to be described, the handling schedule varied in minor ways from one set of experiments to the next; the details of the procedure used will be given in each particular case. Under these conditions, the average time from egg to adult is greatly attenuated and averages about 32 days. Allowing approximately ten days for attainment of sexual maturity, the generation time may be estimated at six weeks. All weighings of flies were made on a Roller-Smith microtorsion balance which had a capacity of 500 mg.

THE EXPERIMENTS

Laboratory populations having marginal or central geographical origins

Population size and production of the Steelville and Chadron populations: In the experiments reported on in this section, two strains of *D. robusta* have been

used, each of which was ultimately derived from a single pair of wild flies collected at Steelville, Missouri, (Sv), a central location in the range of the species, and Chadron, Nebraska, (C) which is geographically and ecologically marginal. These strains were maintained as two of the controls used in the experiments described in CARSON 1958a and were there designated as Sv-135 and C-66 respectively. In January, 1957, four experimental populations were established, two from Sv-135, hereafter referred to as S-1 and S-2 and two from C-66, referred to as C-1 and C-2.

The gene arrangement frequencies present in both of these strains were determined at the time of establishment of the laboratory strains from the F_1 produced by the pair of wild founders and at intervals thereafter (Tables 1 and 2). It will be noted on Table 1 that at the time that S-1 and S-2 were established (early in 1957) gene arrangement 2L-2 had already been lost, leaving 2L and 2L-3 as the two alternative arrangements in the left arm of chromosome 2. The X chromosome is homozygous for gene arrangement; the alternative gene arrangements present in the right arm of the second chromosome are 2R-1 and, in the right arm

TABLE 1

Gene arrangement frequencies (in percent) in the control laboratory populations (S-1; S-2) originally derived from a single pair of wild flies (Sv-135) captured at Steelville, Missouri

Gene arrangement	Aug. 1955 (F_1 from Sv-135)	Dec. 1955	Feb. 1956	July 1956	Jan. 1957 (S-1)	Feb. 1957 (S-2)	Aug. 1957 (S-2)	Nov. 1957 (S-2)	Aug. 1958 (S-2)
			(from culture)						
XL:XR-1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2L	35.0	55.0	70.0	65.0	62.2	80.0	67.3	80.0	70.3
2L-2	25.0	10.0	...	...	...	...	...	...	...
2L-3	40.0	40.0	30.0	35.0	37.8	20.0	33.7	20.0	29.7
2R (2R-1)	75.0	85.0	55.0	45.0	87.9	80.0	50.0	46.7	57.4
3R (3R-1)	45.0	50.0	55.0	65.0	36.5	55.0	30.8	16.7	8.1
Number of chromosomes examined	20	20	20	20	74*	20	52	30	148

* 57 X chromosomes.

TABLE 2

Gene arrangement frequencies (in percent) in the control laboratory populations (C-1; C-2) originally derived from a single pair of wild flies (C-66) captured at Chadron, Nebraska

Gene arrangement	Aug. 1955 F_1 from pair C-66	Jan. 1957 (from culture)	Dec. 1957 (C-2)	Aug. 1958 (C-2)
XL-1:XR	100.0	100.0	100.0	100.0
2L-3:2R	100.0	100.0	100.0	100.0
3R	100.0	100.0	100.0	100.0
N^x	20	19	42	77
N^a	20	20	42	78

N^x = Number of X chromosomes examined.
N^a = Number of autosomes examined.

of the third chromosome, 3R-1. The founder strain and populations C-1 and C-2 have been homozygous for gene arrangement since their inception (Table 2).

Each population was founded from 50 females and 50 males taken at random from the stocks. The change and yeast cycle was precisely as described in CARSON 1961 for *Drosophila melanogaster*. After approximately 12 weeks the populations showed signs of reaching a maximum size and weekly measurements of the size of the adult population (number and weight of individuals present) were begun (see Week 1, Table 3). The figures in Table 3 represent the average of two measurements each week. The starting size (including new additions made on that day) is added to the finishing size seven days later and the sum divided by two (see CARSON 1961). These measurements were carried out for 20 consecutive weeks, during which time each population was handled in identical fashion. The data show that the mean number and weight are higher in S-1 and S-2 than in C-1 and C-2. In only one instance, however, is the difference between two of these means significant at the five percent level. Thus the mean number of flies

TABLE 3

Population size of four experimental populations of Drosophila robusta. *Chadron-1 and -2 (C-1, C-2) are replicates which are homozygous for gene arrangement; Steelville-1 and -2 (S-1, S-2) are replicates which carry a number of inversions*

Week number	Chadron-1 (C-1)			Chadron-2 (C-2)			Steelville-1 (S-1)			Steelville-2 (S-2)		
	No.	Wt. mg	Avg. fly size mg	No.	Wt. mg	Avg. fly size mg	No.	Wt. mg	Avg. fly size mg	No.	Wt. mg	Avg. fly size mg
1	273	498	1.82	261	477	1.83	298	484	1.63	263	462	1.76
2	293	528	1.80	365	609	1.67	305	491	1.61	383	609	1.59
3	354	620	1.75	424	678	1.60	345	558	1.62	446	694	1.56
4	385	651	1.69	469	750	1.60	388	626	1.62	490	766	1.57
5	383	659	1.72	456	711	1.56	405	659	1.63	441	674	1.53
6	367	643	1.75	346	563*	1.63	422	680	1.61	336	528*	1.57
7	365	646	1.77	230	388*	1.69	415	667	1.61	239	400*	1.67
8	369	645	1.75	283	460	1.63	372	613	1.65	322	532	1.65
9	303	529*	1.75	305	489	1.61	305	524*	1.72	345	544	1.58
10	231	413*	1.79	302	498	1.65	235	405*	1.72	320	520	1.63
11	230	392	1.74	284	482	1.70	253	403	1.59	326	536	1.64
12	256	443	1.73	266	456	1.71	253	425	1.68	360	578	1.61
13	340	541	1.59	274	456	1.66	354	544	1.54	367	583	1.59
14	296	452	1.53	330	506	1.53	352	510	1.45	377	594	1.58
15	250	416	1.66	408	585	1.43	332	510	1.54	398	596	1.50
16	336	466	1.39	411	589	1.44	380	598	1.57	394	548	1.39
17	335	517	1.54	337	497	1.48	406	626	1.54	340	465	1.36
18	364	559	1.54	280	419	1.49	386	585	1.52	314	437	1.39
19	322	481	1.50	307	456	1.45	346	534	1.51	291	434	1.49
20	272	411	1.51	326	501	1.54	263	417	1.59	289	472	1.63
Mean weekly number		316.2±11.4			333.2±15.3			340.8±13.1			352.1±14.0	
Mean weekly weight		525.5±20.6			528.5±21.8			542.5±19.8			548.6±20.8	

* Population arbitrarily reduced at one point during the week to exactly 400 mg.

in C-1 differs from S-2, giving a t value of 1.98 with 38 degrees of freedom. The means and their standard errors, however, are affected by the following events. At one point in its 20-week history, each population was reduced artificially to exactly 400 mg wet weight for two consecutive weeks, after which it was again allowed to seek its maximum under natural selection. Furthermore, all populations show a progressive decline in size over the 20-week period, a fact which affects the usefulness of the mean value.

The total number and weight of flies hatched each week ("production") from each of the four populations is given on Table 4. Again, populations S-1 and S-2 outperform C-1 and C-2. The means for weight produced by C-1 and C-2 are very close, a fact which correlates with their closeness in population size. The higher of these two, C-1, differs from the lower Steelville population, S-1, ($t = 2.73$; P is less than .01). It is noteworthy also that S-2 is higher than S-1 ($t = 3.76$; P is less than .001). Differences in number of flies produced are not

TABLE 4

Weekly production of four experimental populations of Drosophila robusta. *Chadron-1 and -2 (C-1, C-2) are replicates which are homozygous for gene arrangement; Steelville-1 and -2 (S-1, S-2) are replicates which carry a number of inversions*

	C-1		C-2		S-1		S-2	
Week number	No.	Wt. mg	No.	Wt. mg	No.	Wt. mg	No.	Wt. mg
1	94	151	125	187	106	165	154	232
2	80	128	121	173	100	164	169	256
3	77	127	101	153	104	168	115	188
4	83	137	70	111	96	163	81	136
5	77	139	60	101	105	160	95	157
6	83	135	116	171	115	187	108	186
7	80	127	85	128	46	110	117	205
8	51	90	67	112	53	90	133	220
9	55	105	69	120	55	93	122	216
10	59	99	60	103	92	143	123	211
11	79	133	27	47	83	146	130	211
12	104	174	51	81	113	184	92	164
13	86	129	95	107	109	174	134	224
14	81	137	164	223	115	185	191	301
15	105	165	122	179	151	232	162	238
16	92	145	80	123	131	204	102	157
17	131	201	82	132	120	215	130	218
18	123	172	113	166	138	204	126	206
19	116	158	83	137	121	174	123	212
20	94	131	111	188	114	179	105	191
Total number	1750		1802		2067		2152	
Mean weekly number	87.5±4.8		90.1±7.2		103.4±19.3		107.6±16.1	
Total weight	2783		2743		3340		4129	
Mean weekly weight	139.2±5.9		137.2±9.5		167.0±8.3		206.5±8.3	

statistically significant at the five percent level of probability. It therefore appears that the Steelville populations, especially replicate S-2, are both larger and more productive than the Chadron populations.

Mass hybridization of a Steelville and a Chadron population: In August, 1957, the adult populations of C-1 and S-1 were discarded and two new hybrid adult populations built up by combining hatching flies from C-1 equally with hatching flies from S-1 continually over a period of about six weeks. These new populations were designated C1S1-A and C1S1-B. C-2 and S-2 were retained as controls.

The production of these populations was measured for 55 consecutive weeks following the completion of hybridization (Table 5). Various population conditions were maintained throughout this period but each of the four populations was treated in identical fashion. For the first 13 weeks, the size of the adult populations was allowed to be determined strictly by natural selection, as in the experiments prior to hybridization (except for one two-week trial period of artificial reduction to 400 mg). During weeks 14–27, however, a ceiling of 425 mg was artificially imposed on the size of each of the four populations. This was accomplished by adding hatching flies to the population twice a week as usual, but adding each time only enough young flies to bring the total weight of the adult population to exactly 425 mg. It was soon found, however, that this level was rather high and that several populations (e.g. C-2 and C1S1-A) usually remained below it. Accordingly, from week 28–55, all populations were artificially held to 300 mg. Under this system, at only one point during the final 20-week period did any population fall naturally below 300 mg during an entire week. This population, C-2, during week 48, fell to a low point of 274 mg even after all additions were made. During all other weeks in all populations, discards were necessary to keep the populations down to 300 mg.

TABLE 5

Production (in mg wet weight/week) of populations of Drosophila robusta *before and after mass hybridization of two populations. After the first 20 weeks, C-1 and S-1 were halved and recombined to make populations C1S1-A and C1S1-B*

	Before hybridization		After hybridization			
	Period 1 Feb. to July 1957 (free population size)		Period 2 Aug. 1957 to Apr. 1958 (various population sizes)		Period 3 Apr. 1958 to Sept. 1958 (pop. size held to 300 mg.)	
Population number	Total weight produced (mg)	Mean weekly weight (mg)	Total weight produced (mg)	Mean weekly weight (mg)	Total weight produced (mg)	Mean weekly weight (mg)
C-1	2783	139.2±5.9	....	.....	....	.....
C-2	2743	137.2±9.5	4000	114.2± 8.9	2112	105.6±15.3
S-1	3340	163.8±8.7	....	.....	....	.....
S-2	4129	206.5±8.3	6137	175.3±10.7	3930	196.5± 8.6
C1S1-A	....	.....	5361	153.2± 9.9	3249	162.5± 7.9
C1S1-B	....	.....	5373	153.5± 9.9	3491	174.6± 7.4
Number of weeks at equilibrium	20		35		20	

Table 5 gives the mean production of the four populations after hybridization; the first two columns in the body of the table repeat the means from Table 4 for comparison. It will be noted that during both periods 2 and 3 (after hybridization), C-2 is the lowest and S-2 is the highest. The hybrid populations are intermediate in value and in one instance, C1S1-A is significantly lower than S-2 at the five percent level or below (S-2 *vs.* C1S1-A, Period 3: $t = 2.912$, P is less than .01).

When populations C-1 and S-1 were combined to form the new hybrid populations C1S1-A and C1S1-B, the question arises whether the gene arrangements introduced from the two sources become balanced or whether one set will replace the other. The results of two tests for the presence of chromosome arrangements in each of these populations one year after the hybridization (approximately nine generations) are given in Table 6. From these data it will be noted that whereas the parental populations are homozygous for different gene arrangements on both arms of the X chromosome (Tables 1 and 2), the hybrid populations display a condition in which X chromosome gene arrangements from both geographical sources have remained in substantial frequency. XL and XR-1 from Steelville are close to 66 percent in each population; this reflects, apparently, a strong tendency for these two right and left arm arrangements to remain associated. Conversely, arrangements XL-1:XR from the Chadron population are similarly associated. Heterozygosity in 2L, 2R and 3R is maintained despite the admixture of homozygous Chadron flies. It is not possible to tell if any intergeographic heterozygotes for these autosomes are maintained in the populations in a manner comparable to that found in the X chromosome.

The facts brought out by the above experiments may be summarized in the following statement. Steelville populations from the center of the range of the species, and carrying a number of inversions, significantly outproduce structurally homozygous Chadron populations from the margin of the range. The sizes of the Steelville populations, furthermore, are likewise somewhat greater. Populations formed by mass hybridization of two of the replicates show a performance

TABLE 6

Gene arrangement frequencies (in percent) in two experimental laboratory populations (C1S1-A; C1S1-B) one year after they were formed by mass hybridization of Chadron population C-1 and Steelville population S-1

Gene arrangement	C1S1-A Aug. 1958	C1S1-B Aug. 1958
XL	68.1	67.3
XL-1	31.9	32.7
XR	34.8	35.5
XR-1	65.2	64.5
2L	26.2	45.2
2R	75.4	69.2
3R	55.0	52.8
N	104	69

close to that of the Steelville control population and in no case surpass it, whereas the Chadron control population continues to be the lowest. Despite their failure to exceed the performance of the Steelville populations, however, the hybrid populations have considerably more chromosomal polymorphism than the Steelville control because in these populations both left and right arms of the X chromosome carry intergeographical polymorphism.

Performance in "open" and "closed" populations

In August, 1956, a large sample of *Drosophila robusta* was obtained from Plainwell, Michigan (CARSON 1958b). Gene arrangement frequencies were obtained by studying the chromosomes of larvae which were F_1's from the wild individuals. The results of this study are shown in the first column in the body of Table 7. Individual pairs nos. 1 through 18 were chosen and an experimental population (PW) started using all 36 adults and their F_1's. This population came to equilibrium and was handled in an identical manner to the populations in the previous experiment (S-1, S-2, C-1 and C-2). Size and production measurements made over a 20-week period in early 1957 are given in Table 8. It will be noted that this performance is very similar to the performance of the Steelville populations previously described (Tables 3 and 4). During this time all polymorphism present at the beginning of the experiment was retained except for 3R-1 and 3L-R which were apparently eliminated (Table 7, 3rd column).

In July of 1957, PW was divided into two populations (PW-A and PW-B) and each replicate was allowed to come to equilibrium. Beginning immediately at the time that the split was made, and continuing thereafter for 31 consecutive

TABLE 7

Gene arrangement frequencies (in percent) in samples from a natural population of D. robusta *from Plainwell, Michigan (PW) and in open (PW-A received 82 wild males over a 31-week period) and closed (PW-B) laboratory populations derived from it*

Gene arrangement	F_1 from wild population		Experimental laboratory populations				
	Total sample Aug. 1956	Pairs 1–18 Aug. 1956	(closed) PW Mar. 1957	(open) PW-A Aug. 1957	(closed) PW-B Aug. 1957	(open) PW-A Dec. 1957	(closed) PW-B Dec. 1957
XL	44.9	38.9	52.6	72.5	50.0	56.1	45.9
XL-1	55.1	61.1	47.4	27.5	50.0	43.9	54.1
XR	21.2	19.4	49.6	51.7	64.7	31.8	26.2
XR-1	76.3	75.0	50.4	48.3	35.3	68.2	73.8
2L	57.6	52.8	45.6	20.7	11.4	29.8	5.2
2L-1	5.9	5.5	30.9	47.9	63.6	41.7	57.1
2L-2	8.5	16.7	8.1	17.1	13.6	10.7	29.9
2L-3	28.0	25.0	15.4	14.3	11.4	17.8	7.8
2R	89.8	80.6	94.0	87.1	86.3	85.7	89.8
3R	94.1	91.7	100.0	95.0	100.0	86.9	100.0
3R-1	4.2	5.5	...	5.0	...	13.1	...
3L-R	1.7	2.8	...	...	...	...	...
N^x	118	36	133	120	34	66	61
N^a	118	36	136	140	44	84	77

TABLE 8

Population size and production of experimental populations derived from Plainwell, Michigan. PW and PW-B are closed populations, whereas PW-A received 82 wild males over a 31-week period

Population designation	Period	Population size mg wet wt./week	Production Total	Production mg wet wt./week
PW	20 weeks Feb.-July 1957	548.0±16.5	2989	149.6±12.0
PW-A (open)	26 weeks Aug. 1957-Feb. 1958	472.6±17.6	3899	150.0±11.2
PW-B (closed)	same as above	503.2±14.5	4094	157.5±12.3

weeks, wild-type males from various sources were added to PW-A. No additions were made to PW-B. The males were added to PW-A one at a time, usually each time the population was opened for weighing and counting. For the first six weeks, four males per week were added; from the seventh through the 31st weeks, five males per week were added. In order of addition, the number and source of the males was: Olivette, St. Louis County, Missouri (flies came directly from nature) 57; scarletoid net (laboratory mutants) 8; C1S1-A (see previous section) 2; Springfield, Ohio (directly from nature) 60; Webster Groves, Missouri (directly from nature) 22.

Over the 26-week period that the introductions were made into PW-A, population size and production were measured (Table 8). The differences between the means are not significant (e.g. population size of PW-B *vs.* PW-A gives a t value of 1.342; $P = 0.5$ to 0.10). Thus it is apparent that the introduction of foreign flies has not been associated with a corresponding increase in population size.

Gene arrangement frequencies in the original PW population and in the replicates derived from it are shown in Table 7. It will be noted that whereas PW and the closed derivative from it, PW-B, have 100 percent 3R, PW-A shows 3R-1 in substantial frequency. This gene arrangement clearly was introduced into this population with some of the wild males. As in the previous experiment, experimental populations of *D. robusta* seem to be highly tenacious of chromosomal polymorphism.

To summarize the facts: an experimental population with many gene arrangements from Plainwell, Michigan, is very similar in performance to populations from Steelville, Missouri. This performance is not altered by the introduction of a continual flow of males from different sources and geographical areas despite the fact that at least some of these introductions have been proved to be successful.

Gene arrangement frequencies in closed populations under strong natural selection

In July and August, 1957, collections were made at Bridgewater, Vermont, and Olivette, Missouri, respectively. The results of the analyses of the chromosomes from the wild females are given in the first and fourth columns in the body

of Table 9. The Vermont sample shows typical marginal characteristics, as has been pointed out in CARSON (1958a,b). The Olivette sample came from Olivette Woods (CARSON 1958b) and shows, 11 years after the first study of this locality, the same equilibria which have been observed throughout the period of study. The only apparent difference is the high level of 2L relative to 2L-1. These two gene arrangements, however, have made noteworthy fluctuations in the past but have always returned later to intermediate levels.

Immediately following capture of the wild flies, two Bridgewater (B-1 and B-2) and two Olivette (O-1 and O-2) populations were established. Each Bridgewater population was begun in August, 1957 using two females and two males from each of 48 F_1's from wild females. Thus a total of 192 founder flies were used for each replicate. The Olivette populations were also begun in August using two females and two males from each of 50 F_1's from wild females. A total of 200 founder flies were thus used for each replicate.

All four of these populations were maintained on a schedule identical to that described previously until equilibrium size of approximately 500 mg or more was achieved. At this point, in November of 1957, a new cycle of change was instituted and measurements of size and production were abandoned. A new vial with food and 10 mg yeast was added only once a week; at the same time, collection of hatching flies was made from all vials which had previously been in contact with the population and these flies added to the adult population. Under these

TABLE 9

Gene arrangement frequencies (in percent) in samples from various natural populations of D. robusta *and in experimental populations derived from them after 30 generations of strong natural selection*

Gene arrangement	Bridgewater, Vermont, F_1 from wild population July 1957	Laboratory populations		Olivette, Missouri, F_1 from wild population Aug. 1957	Laboratory populations	
		B-1 May 1960	B-2 Apr. 1960		O-1 May 1960	O-2 May 1960
XL	2.9	31.3	48.6	99.5	100.0	100.0
XL-1	97.1	68.7	51.4	...	...	...
XL-2	...	...	...	0.5	...	...
XR	95.4	100.0	100.0	40.3	53.3	36.7
XR-1	...	...	...	56.0	46.7	63.3
XR-2	4.6	...	...	3.7	...	...
2L	26.1	18.6	30.9	70.8	31.9	50.6
2L-1	8.9	35.1	53.0	21.3	57.5	22.8
2L-2	...	...	...	6.9	10.0	21.5
2L-3	65.0	46.4	16.0	0.9	...	...
2L-8	...	...	...	...	...	5.1
2R	100.0	100.0	100.0	83.3	93.1	98.1
3R	98.8	100.0	100.0	77.8	59.4	85.4
3R-1	0.4	...	...	22.2	40.6	14.6
3L-R	0.8	...	...	...	...	...
N^x	174	83	142	216	160	158
N^a	257	97	181	216	120	120

conditions, the equilibrium size of the population was reduced to approximately 125 mg. This treatment was continued for approximately two and one half years, about 30 generations, after which time chromosome samples were taken by removing individuals or pairs of flies as they hatched under strong natural selection and testing them for the presence of chromosome aberrations. The results of these tests are given in Table 9.

There appears to be a tendency for loss of gene arrangements which were rather rare in the founders. Thus, XR-2 is lost from both Bridgewater and Olivette populations, 2L-3 is lost from the Olivette populations and 3R-1 and 3L-R are lost from the Bridgewater populations. Polymorphism is nonetheless retained tenaciously in a number of instances; the levels of polymorphism are in a number of cases different in the different replicates.

Of particular interest is the appearance of a new gene arrangement, 2L-8, in population O-2. This arrangement appears to have arisen within this population and has achieved a frequency of around five percent. The new inversion is a very short one. Its distal break is near the base of the arm, in the middle of region 23A (CARSON 1958b). This region consists of six bands and the break is between bands number three and four. The proximal break is in the heterochromatin, that is, its position is indistinguishable in the salivary gland chromosome from the position of the proximal breaks of 2L-2 and 2L-3. There is no evidence that the inversion is pericentric. Inversion 2L-8, furthermore, is associated with a gene arrangement which otherwise has the 2L-1 gene arrangement; it is probable, therefore, that it arose in and has remained associated with this arrangement.

DISCUSSION

The relatively high fitness of the Steelville and Plainwell populations under the test conditions appears to be correlated with the high degree of chromosomal polymorphism that they contain. In a discussion of this type of genetic adjustment, the writer (CARSON 1959) suggested that this polymorphism may permit the populations concerned to exploit the environment through "heterotic buffering", that is, the supergene heterozygotes are more fit than homozygotes because of their better general performance in all niches of the environment and all phases of the life of the individual. This interpretation applies particularly to large central rather than small marginal populations. The latter are considered to be inbred, rather closely adapted to a limited number of niches, specialized genetically and relatively homozygous. Experimental populations of marginal origin (e.g. Chadron, Nebraska) perform less well under the test conditions than populations of central origin.

When the relatively low-fitness marginal populations from Chadron, Nebraska were hybridized with the relatively high-fitness central populations from Steelville, Missouri, the resulting hybrid populations do not differ in relative fitness from the parental Steelville populations. In other words, heterosis is not observed on the populational level. A like result is obtained when attempts are made to improve the Plainwell, Michigan, population by opening it to a continual inflow of new genetic material.

The proposal may be made that in the two instances mentioned above the populations of central origin are already carrying a high degree of heterozygous balance and may be very nearly saturated in the sense that little improvement by increasing the amount of heterotic buffering is possible. The fact that further polymorphism is nonetheless accepted in chromosome X (for the Chadron-Steelville hybrid populations) and chromosome 3 (for the open Plainwell population) while no further increase in population size or production is observed may be explained as follows. We may assume that saturation is not complete but that the increment of change in population fitness is so small as not to be detectable under these circumstances and by the method used. Evidence for "saturation" of a heterotic system was presented by CARSON (1961) who showed that several populations of *D. melanogaster*, which were carrying considerable polymorphism would not readily accept more.

The Steelville populations ultimately were derived from a single pair of wild flies whereas the Plainwell populations were established from 18 pairs. This difference in genetic background, however, does not appear to affect the final performance of these two types of populations. They are almost identical in this regard. It is suggested that the considerable heterozygosity carried by the original pair of wild Steelville flies is correlated with the high performance of the populations derived from it. This may be regarded as indicative of the great storage capacity of the heterozygous karyotypes found in central populations.

Most of the gene arrangements present in experimental populations of *D. robusta* which are polymorphic are retained for long periods, perhaps indefinitely. This is true also of the Bridgewater and Olivette populations which were carried for about 30 generations under very severe selection. This persistence is considered to be due to the large role played by the heterotic buffering system of the organism which suddenly finds itself facing an uniquely new environment such as that provided by the experimenter in the laboratory. The data nevertheless show that in a number of instances gene arrangements which were originally rare in the sample when it was taken from nature are lost from the experimental populations derived from these samples. This may be due to the relatively small size of the experimental populations. This should permit chance fixation in the population by random drift of those gene sequences which have homozygotes which are very close in selective value to the pertinent heterozygote. The large size of natural populations would be expected to prevent such fixation in central populations. XL-2, XR-2 and 2L-3, for instance, have been present in the Olivette, Missouri, population for 11 consecutive years at frequencies which often fall below one percent, yet are consistently encountered in population samples (CARSON 1958b). Experimental populations established from this area, however, show loss of these "rare" arrangements.

The case where a new arrangement arose in an experimental population and increased in frequency therein requires comment. The small inversion involved was at the base of the second chromosome; specifically, the change occurred in arrangement 2L-1. This latter arrangement existed in the population in high

frequency, apparently balanced there by heterosis, at the time of the occurrence of the new inversion. Because of the well-known suppressive effects of inversions on crossing over in *D. robusta* (CARSON 1953; LEVITAN 1955) separation of this new inversion from the arrangement in which it arose would be expected to be rare and was, in fact, not observed.

The selective relationships between the various gene arrangements in this chromosome arm would be expected to be complex, as the addition of the new arrangement raises the number of coexisting gene arrangements to four and the number of competing karyotypes for this arm to ten, four homozygotes and six heterozygotes. This new gene arrangement could have increased in frequency either because it conferred some selective advantage on those individuals carrying it or because its increase was largely fortuitous due, at least partly, to linkage with a gene arrangement already participating in a major heterotic association. This latter possibility is further increased by the fact that the population was small throughout the experiment. In a more extensive analysis of a similar case SPERLICH (1959) demonstrated that a new X-ray-induced inversion in *D. subobscura* behaved in a heterotic manner in three different population cages in which it was tested with different alternative sequences. In the absence of similar tests, no decision can be made concerning this new aberration in *D. robusta*.

There appears to be no general and progressive tendency for decrease in chromosomal polymorphism in the relatively uniform environment in which these laboratory populations were maintained. In these long-term experiments, and in previous ones (LEVITAN 1951; CARSON 1958b) the reduction of variability observed by LEWONTIN (1958) was not observed. The writer feels that in *D. robusta* the sectional heterozygotes have some general physiological homeostasis which provides for heterotic buffering even in the relatively, but by no means absolutely, uniform conditions under which the populations are reared. LEWONTIN's hypothesis that polymorphism should be lost in a uniform environment, furthermore, would appear to hold only if each heterozygote is specially adapted in nature to some slightly different environmental variable which is not recapitulated in the laboratory conditions.

SUMMARY

1. Laboratory populations of *Drosophila robusta* have been maintained under continual strong natural selection with carefully controlled conditions of food and space in such a way as to provide an opportunity for weekly measurement of population size (weight and number of adult individuals) and production (weight and number of individuals hatched).

2. Experimental populations derived from the center of the species range tend to remain highly polymorphic for gene arrangement throughout the experiments. Their performance is better than structurally homozygous lines derived from marginal sites.

3. Populations derived from hybridization of marginal and central populations do not surpass the performance of the parental populations of central origin. They nevertheless retain much polymorphism, some of which is intergeographic.

4. Experimental populations kept genetically open by continual additions of foreign flies did not detectably surpass those to which no additions were made, although it was proved that at least one intergeographic polymorphism was added under natural selection.

5. These facts suggest that, within the limits of the sensitivity of the method for measuring population fitness, the original (central) polymorphic populations are close to saturation and cannot, at least under the conditions of population size obtaining in the experiments, be significantly improved by the addition of further polymorphism.

6. Experimental populations derived from one pair of flies from the center of the range and populations derived from many pairs of flies both showed a high degree of performance and were not different from one another. This suggests a high storage capacity in karyotypes from central populations.

7. Populations under extreme adverse conditions, wherein the food was very closely limited and population size remained small, nevertheless retain their polymorphism tenaciously over many generations. The frequencies of the various arrangements, however, achieve equilibria different from the wild populations from which they came. In some instances, furthermore, they are different from each other.

8. A new inversion arose within one population and under natural selection rose to a frequency of about five percent. It is suggested that this new aberration may be quasi-neutral and may have been carried along because it is closely linked to a gene arrangement which exists in the population in balanced condition.

9. These findings generally provide support for the hypothesis that chromosomal polymorphism, as found in central populations, provides heterozygotes which are superior to their corresponding homozygotes in all facies of the environment, that is, show heterotic buffering. In contrast to this, marginal populations appear to be rather more narrowly specialized and exploit the environment through fixation of genes or gene combinations in the homozygous state.

ACKNOWLEDGMENTS

I wish to thank Mrs. Carole Konrad, Mrs Koyoko Kato and Miss Jannette Corn for their painstaking assistance with the routine handling of the experimental populations. Collections of flies from Webster Groves, Missouri, Plainwell, Michigan, and Springfield, Ohio, were made by Dr. H. D. Stalker, to whom I am greatly indebted not only for this but for many other kindnesses. This work was supported by grants G-421 and G-7441 from the National Science Foundation.

LITERATURE CITED

Bert, G. R., 1960 Fixed heterozygosity and fitness in *Drosophila melanogaster* populations under strong natural selection. Genetics **45**: 975.

Buzzati-Traverso, A. A., 1955 Evolutionary changes in components of fitness and other polygenic traits in *Drosophila melanogaster* populations. Heredity **9**: 153–186.

CARSON, H. L., 1953 The effects of inversions on crossing over in *Drosophila robusta*. Genetics **38**: 168–186.

1955 The genetic characteristics of marginal populations of Drosophila. Cold Spring Harbor Symposia Quant. Biol. **20**: 276–287.

1958a Response to selection under different conditions of recombination in Drosophila. Cold Spring Harbor Symposia Quant. Biol. **23**: 291–306.

1958b The population genetics of *Drosophila robusta*. Advances in Genet. **9**: 1–40.

1958c Increase in fitness in experimental populations resulting from heterosis. Proc. Natl. Acad. Sci. U.S. **44**: 1136–1141.

1959 Genetic conditions which promote or retard the formation of species. Cold Spring Harbor Symposia Quant. Biol. **24**: 87–105.

1961 Heterosis and fitness in experimental populations of *Drosophila melanogaster*. Evolution **14**: (in press).

LEVITAN, M., 1951 Experiments on chromosome variability in *Drosophila robusta*. Genetics **36**: 285–305.

1955 Studies of linkage in populations. I. Associations of second chromosome inversions in *Drosophila robusta*. Evolution **9**: 62–74.

LEWONTIN, R. C., 1958 Studies on heterozygosity and homeostasis. II. Loss of heterosis in a constant environment. Evolution **12**: 494–503.

L'HÉRITIER, P., Y. NEEFS, and G. TEISSIER, 1937 Aptérisme des insectes et sélection naturelle. Compt. rend. (Paris) **197**: 1765–1767.

REED, S. C., and E. W. REED, 1948 Natural selection in laboratory populations of Drosophila. Evolution **2**: 176–186.

SMATHERS, KATRINA M., 1961 The contribution of heterozygosity at certain gene loci to fitness of laboratory populations of *Drosophila melanogaster*. Am. Naturalist **95**: 27–38.

SPERLICH, D., 1959 Experimentelle Beitrage zum Problem des positiven Heterosiseffektes bei der Strukturpolymorphen Art *Drosophila subobscura*. Z. Vererb. **90**: 273–287.

WRIGHT, S., and TH. DOBZHANSKY, 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. Genetics **31**: 125–136.

EFFECTS OF FLOWER NODE POSITION ON THE MUTABLE V AND STABLE R LOCI IN A CLONE OF NICOTIANA

SEAWARD A. SAND

Genetics Department, The Connecticut Agricultural Experiment Station, New Haven, Conn.

Received December 19, 1960

GENETIC analysis implying mutable alleles of the gene *V* to account for flower color variegation in a Nicotiana culture has been presented by SMITH and SAND (1957). These plants also contain stable genes for flower color and have been found useful for the study of interaction between environmental factors and genetic systems of varying stability (SAND 1957; SAND, SPARROW and SMITH 1960). The goal has been to elucidate differences and similarities between mutable and stable gene systems.

Data are presented here for both somatic and germinal effects due to flower node position on a mutable allele of the *V* gene and on the stable *R* gene in a clone of Nicotiana. Results for flowers from various node positions are not homogeneous, but vary according to a pattern that is related to the sequence of node positions on the main flowering axis. It is inferred that metabolic changes associated with development of the branch, and correlated with growth rate, influence the mutation rates of both the stable and the mutable genes. This work has been summarized previously (SAND 1960).

MATERIALS AND METHODS

Nicotiana clone S5230-5, selected from cultures of interspecific hybrid origin, was employed here as used previously in studies of temperature and chronic gamma irradiation effects on mutation. Flowers of this clone are illustrated and detailed procedures appear in the earlier papers, cited above. Only general methods and limited details will be outlined here.

Replicated cuttings of the clone were grown in sand culture with a standard nutrient solution. Banks of fluorescent lights were operated to provide a 14-hour day, and the room temperature was automatically controlled at 68°F ± 2°.

The clone is heterozygous for the mutable *V* gene (v_{83}/v_s) and for the stable *R* gene (R/r), which affect flower color. The somatic effect on a mutable gene system was compared with a stable gene system in the same tissue by counting speckled sectors (changes from v_{83} to v_s phenotype) and purple sectors (changes from *R* to *r* phenotype) on flowers from successive nodes of the main flowering axis of each plant. Similarly, germinal effects on the same gene systems were studied by counting speckled and purple individuals in progeny obtained by self-pollinating flowers at selected node positions.

Figure 1 presents graphically the somatic response obtained for sector frequency as a function of node position. The 222 scored flowers came from 24

RESULTS

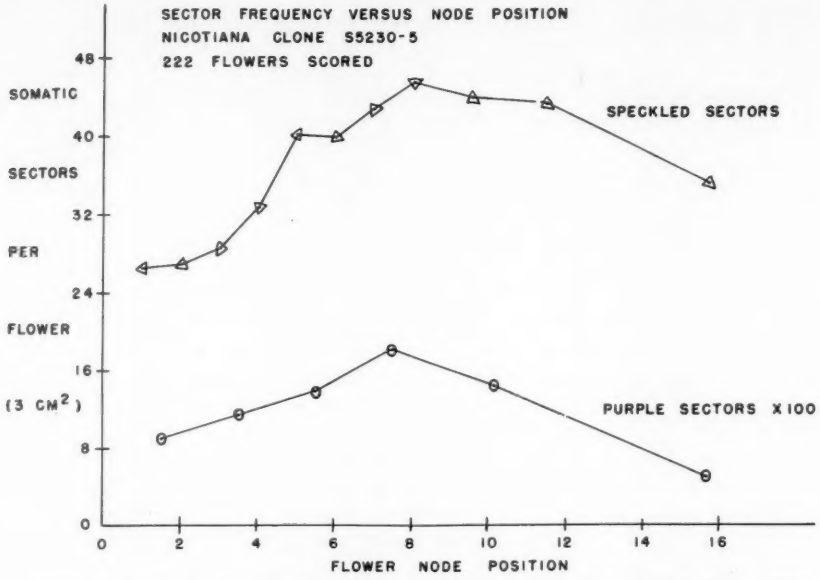


FIGURE 1.—Response curves showing the frequencies of somatic sectors on flowers from successive node positions. Purple sectors (associated with the stable locus *R*) are about 300 times less frequent, but respond in a pattern similar to that for speckled sectors (associated with the mutable locus *V*).

different flowering branches and cover a total range of node positions from one to 21. Data for all members of the clone were pooled within node position groups. Each point plotted represents an average for 16 or more flowers. The purple sector frequency has been graphed on a scale 100 times larger than that for speckled sectors. Actually, purple sectors are nearly 300 times less frequent than speckled sectors, providing a phenotypic basis for designating *R* as a stable gene and v_{83} as mutable. The average somatic sectoring rates for both the mutable and stable genes vary with node position in a manner that suggests direct correlation with the natural change in rate of growth of the flowering branch. Sector frequencies at mid-branch are about twice as high as for proximal and distal nodes.

Table 1 summarizes an analysis of variance of the speckled-sector sums for flowers pooled in three node-position groups: low (nodes 1–6), middle (nodes 7–12), and high (nodes 13–21). The average sector frequency for the low nodes (32) is not significantly different from that for the high nodes (35). However, the average sector frequency for the middle nodes (43) is significantly greater than the average for the other two nodal groups.

TABLE 1

Analysis of variance of speckled-sector sums for flowers from low, middle and high node positions

Source	d.f.	Sum of squares	Mean square
Among 3 node positions	2	5,774	2,887**
(Low + high) *vs.* mid nodes	1	5,572	5,572**
Low *vs.* high nodes	1	202	202 n.s.
Error (within node positions)	219	85,100	389
Total (C.F.M.)	221	90,874	. . .

n.s. Not significant at 5 percent level.
** Significant at 1 percent level.

A similar analysis of purple-sector sums fails to show statistically significant differences among the three groups of pooled node positions. The difficulty lies in the extremely low frequency with which purple sectoring occurs. The 222 flowers scored were an adequate sample for speckled sectoring (7,984 total sectors counted), but were an inadequate sample for purple sectoring (27 total sectors counted). Nevertheless, the purple-sector data are not random in distribution, but instead show a pattern of variation with node position similar to that for speckled sectors. The averages for low, middle, and high node groups are respectively 0.11, 0.16, and 0.05 purple sectors per flower.

Progeny segregation was tested by growing plants from self-pollinated seed produced at selected node positions on five members of the clone. This seed was pooled within each of 14 node position groups, distributed from six percent to 96 percent of the total nodal range. The 14 corresponding families containing 1192 individuals were scored for their frequencies of speckled and purple progeny. Table 2 summarizes an analysis of variance for these data obtained after grouping the 14 families into three categories representing low, middle and high node positions. It can be seen that the interaction between node position and progeny type is highly significant. Apparently the node position response for speckled progeny is different from the node position response for purple progeny. The pattern of this interaction is shown in Figure 2. For the frequency of speckled

TABLE 2

Analysis of variance of frequency of speckled and purple progeny from low, middle and high node positions

Source	d.f.	Sum of squares	Mean square
Among 3 node positions	2	62.7	31 n.s.
Speckled *vs.* purple progeny	1	558.0	558**
Node position × progeny	2	283.8	142**
Error	22	332.6	15
Total (C.F.M.)	27	1,237.1	. .

** Significant at 1 percent level.
n.s. Not significant at 5 percent level.

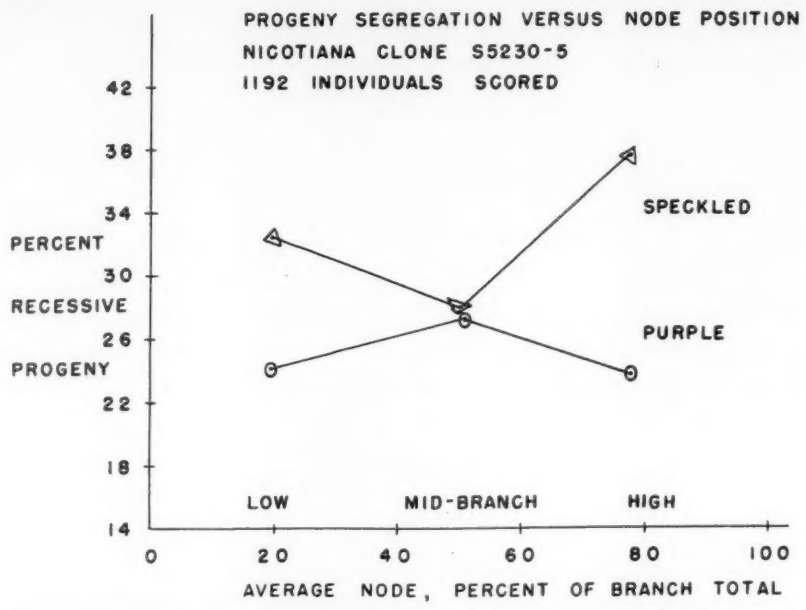


FIGURE 2.—Response curves showing influence due to node position of self-pollinated flowers of the heterozygous parental clone upon the progeny segregations obtained. The frequencies of recessive progeny (speckled and purple) vary inversely for the mutable locus *V* and the stable locus *R*.

progeny an effect due to node position of about ten percent is indicated, with the *minimum* observed in families from mid-branch pollinations. However, mid-branch pollinations give the *maximum* purple progeny frequencies.

DISCUSSION

On the basis of an analysis for the bicolor gourds, SHIFRISS (1955) has proposed a model in which genetic instability conditions developmentally precocious physiological events. These physiological events are prerequisite to extreme fluctuations in phenotype superposed by other genetic and environmental variations. It would follow that part of the normal stable genetic system functions as a buffer against certain potential causes of phenotypic variability external to itself. Such buffering mechanisms are interactions having possible significance for the control of development. WHYTE (1960) has called attention to the theoretical necessity in evolution for developmental selection of genes, where the selective criterion is internal organizational efficiency. One view of mutable genes would consider them normal genetic entities reacting abnormally because of a disorganized relationship with other parts of the chromosomal or cellular system. Mutable gene phenomena would thus represent the failure of integration proc-

esses normal to organisms, illustrating the existence of interaction systems made readily observable by partial loss of their coordination (SAND, SPARROW and SMITH 1960).

With such sensitized genetic systems it is then possible to demonstrate the influence of other factors including internal environment upon expression and reproduction of the system. For example, VAN SCHAIK (1955) has reported studies on the variegated pericarp allele in corn for which a correlation of metabolic vigor with mutation rate seems the best integrating principle for both environmental and hybrid effects that were observed. Also for variegated pericarp in maize, ANDERSON and EYSTER (1928) found an increased rate of mutation as ontogeny of the ear shoot and kernel proceeds. In addition, LEWIS (1953) detected a higher frequency of rogue tomatoes among plants produced by seed from the third and fourth inflorescence in comparison with the first and second inflorescence. The present studies with Nicotiana confirm this type of phenomenon and extend it to show relation between mutable gene response and flower node position throughout the grand period of growth of the branch. The inverse pattern of somatic and germinal response in this case suggests not merely an effect of tissue environment on response, but also a dramatic effect on the direction of the response.

OGUR, OGUR and ST. JOHN (1960) offer a model of differential temperature effect upon cell division *versus* particle division to interpret the temperature dependence of mutation rate to respiration deficiency in Saccharomyces. Lower relative rates of particle reproduction at low and at high temperatures increase the probability of particle loss and thus increase the rate of mutation. The possibility exists in other systems for alteration of chromosomal differentiations or alteration of the statistical probability of exact genic reproduction by metabolic conditions related to growth and development. That reactions affecting heritable alterations in genetic material are not independent of normal physiological cycles is also indicated by work of OTTOLENGHI and HOTCHKISS (1960), who have found that the rate of genetic transformation in mixed Pneumococcal cultures is related to the stage of growth of the culture.

BRINK (1960) has summarized a portion of his discussion as follows: "Differential sensitivity to heritable change in varying cellular environments must be considered one of the characteristic features of unstable loci." However, there are indications that this feature is not limited to unstable loci, but may characterize stable loci as well, although to a lesser degree. The somatic response to node position for the stable *R* locus in Nicotiana is parallel to the somatic response shown by the unstable *V* locus. The progeny segregation responses to node position are inverse for the two loci, but heritable response to cellular environment is indicated, nevertheless, for both genes. The evident specific differences are of a sort required by a model accounting for differential activity of genes in part as differential response to developmental stimuli. It is suggested that responses of unstable loci give clues to mechanisms also operative for stable loci and pertaining to interactions significant for differentiation and development.

SUMMARY

Data are presented for somatic and germinal effects due to flower node position on a mutable allele of the *V* gene and on the stable *R* gene both of which affect flower color in a clone of Nicotiana. Replicated cuttings of the heterozygous clone (v_{S3}/v_s, R/r) were grown in sand culture under conditions of controlled temperature and light. The somatic effect on a mutable gene system was compared with a stable gene system in the same tissue by counting speckled sectors (changes from v_{S3} to v_s phenotype) and purple sectors (changes from *R* to *r* phenotype) on flowers from successive nodes of the main flowering axis. Germinal effects on the same gene systems were studied by counting speckled and purple individuals in progeny obtained by self-pollinating flowers at selected node positions.

At mid-branch nodes somatic sectoring rates for both the mutable and stable genes are about twice as high as for proximal and distal nodes. In progeny segregation both genes also respond to node position, but in an inverse pattern: families from mid-branch pollinations give the *minimum* frequency of speckled progeny but the *maximum* frequency of purple progeny. It is concluded that stable genetic loci, as well as unstable loci, may exhibit differential sensitivity to heritable change in varying metabolic or cellular environments.

LITERATURE CITED

ANDERSON, E. G., and W. H. EYSTER, 1928 Pericarp studies in maize. III. The frequency of mutation in variegated maize pericarp. Genetics **13**: 111–120.

BRINK, R. A., 1960 Paramutation and chromosome organization. Quart. Rev. Biol. **35**: 120–137.

LEWIS, D., 1953 The rogue tomato: a problem in nuclear, cytoplasmic and environmental control. Heredity **7**: 337–361.

OGUR, M., S. OGUR, and R. ST. JOHN, 1960 Temperature dependence of the spontaneous mutation rate to respiration deficiency in Saccharomyces. Genetics **45**: 189–194.

OTTOLENGHI, E., and R. D. HOTCHKISS, 1960 Appearance of genetic transforming activity in Pneumococcal cultures. Science **132**: 1257–1258.

SAND, S. A., 1957 Phenotypic variability and the influence of temperature on somatic instability in cultures derived from hybrids between *Nicotiana langsdorffii* and *N. sanderae*. Genetics **42**: 685–703.

1960 Relation between Sachs' grand period of growth and mutation at two loci in Nicotiana. (Abstr.) Genetics **45**: 1009.

SAND, S. A., A. H. SPARROW, and H. H. SMITH, 1960 Chronic gamma irradiation effects on the mutable *V* and stable *R* loci in a clone of Nicotiana. Genetics **45**: 289–308.

SHIFRISS, O., 1955 Genetics and origin of the bicolor gourds. J. Heredity **46**: 213–222.

SMITH, H. H., and S. A. SAND, 1957 Genetic studies on somatic instability in cultures derived from hybrids between *Nicotiana langsdorffii* and *N. sanderae*. Genetics **42**: 560–582.

VAN SCHAIK, T., 1955 Effect of plant vigor on stability of the variegated pericarp allele. J. Heredity **46**: 100–104.

WHYTE, L. L., 1960 Developmental selection of mutations. Science **132**: 954.

SPONTANEOUS SEX REVERSALS OF GENOTYPIC MALES IN THE PLATYFISH (XIPHOPHORUS MACULATUS)[1]

PAMELA A. MAC INTYRE

Genetics Laboratory of the New York Aquarium, New York Zoological Society

Received January 6, 1961

THE platyfish, *Xiphophorus maculatus*, exhibits two types of sex-determining mechanisms: the male is heterogametic (XX female, XY male) in those from Mexico, while the female is heterogametic (WY female, YY male) in those from British Honduras and in most domesticated strains (GORDON 1951). A few instances of spontaneous sex reversal (an individual who is functionally one sex, but genotypically the other) have been reported in this well-studied laboratory fish, most of them in domesticated stocks of unknown ancestry which have been bred by hobbyists or dealers. Such stocks usually include interracial or interspecific hybrids and are thus of questionable value in studies of sex determination. Most reported instances have been single exceptions in otherwise normal broods.

The instances of sex reversal described in this paper are of interest in that two well-studied strains of known wild ancestry that had been maintained by inbreeding in this laboratory for many years produced a number of sex reversals within a period of one and one half years. All the exceptional fish were females that were genotypic males, which is the opposite from other reported sex reversals in this species.

Background of strains 163A and 163B

Strains 163A and 163B are derived from a collection of *X. maculatus* from the Río Jamapa in Mexico made in 1939. Strain 163 was inbred by brother-sister matings for six generations, then separated into two strains: strain A, which carries the *Sd* (spotted dorsal) gene on the X chromosome and the *Sr* (striped side) gene on the Y chromosome; and strain B, which carries the *Sp* (spotted side) gene on the X chromosome and the *Sr* gene on the Y chromosome. These genes are part of a multiple allelic series of dominant sex-linked genes controlling macromelanophore pigment patterns (GORDON 1951). At present, strain 163A has been inbred for 19 and strain 163B for 17 generations. Good evidence for their genetic homozygosity is provided by the fact that all tissue transplants among strain 163 fish are accepted (KALLMAN 1960, personal communication). In strain 163A, all females are $X_{Sd}X_{Sd}$ and the males $X_{Sd}Y_{Sr}$. In strain 163B, the females are $X_{Sp}X_{Sp}$ and the males $X_{Sp}Y_{Sr}$.

Each of these strains is maintained, under conditions described by GORDON

[1] This work was supported by a grant, C-4945, from the National Cancer Institute to DR. SYLVIA GREENBERG and was aided by the laboratory facilities of the American Museum of Natural History, New York 24, New York.

(1950), in two rooms at the Genetics Laboratory. Several crosses may be made in each generation, but only one pair of siblings of each generation is chosen to carry on the inbred line.

Occurrence of sex reversals

Among the fish of strains 163A and 163B born in one of the rooms during the fall of 1958, several females with the genotypic constitution of males were observed (Table 1). A brood of the 12th inbred generation of 163B consisted en-

TABLE 1

Occurrence of sex-reversed XY females in strains 163B and 163A

Parents		Offspring				
Female	Male	Pedigree number	Date born	$X_{Sp}X_{Sp}$ ♀♀	$X_{Sp}Y_{Sr}$ ♂♂	$X_{Sp}Y_{Sr}$ ♀♀ (sex-rev.)
$163B^{11}$–1($X_{Sp}X_{Sp}$)*	$163B^{11}$–11($X_{Sp}Y_{Sr}$)	$163B^{12}$a	8/5/58	8	7	0
$163B^{11}$–1($X_{Sp}X_{Sp}$)	$163B^{11}$–11($X_{Sp}Y_{Sr}$)	$163B^{12}$a	8/26/58	5	9	0
$163B^{11}$–2($X_{Sp}X_{Sp}$)	$163B^{11}$–11($X_{Sp}Y_{Sr}$)	$163B^{12}$b	8/13/58	11	0	9
				$X_{Sd}X_{Sd}$ ♀♀	$X_{Sd}Y_{Sr}$ ♂♂	$X_{Sd}Y_{Sr}$ ♀♀ (sex-rev.)
$163A^{13}$–1($X_{Sd}X_{Sd}$)	$163A^{13}$–11($X_{Sd}Y_{Sr}$)	$163A^{14}$a	10/17/58	5	2	8
$163A^{16}$–1($X_{Sd}X_{Sd}$)	$163A^{16}$–11($X_{Sd}Y_{Sr}$)	$163A^{17}$a	2/1/60	9	7	0
$163A^{16}$–1($X_{Sd}X_{Sd}$)	$163A^{16}$–11($X_{Sd}Y_{Sr}$)	$163A^{17}$a	3/1/60	9	3	1
$163A^{16}$–2($X_{Sd}X_{Sd}$)	$163A^{16}$–11($X_{Sd}Y_{Sr}$)	$163A^{17}$b	2/23/60	4	4	5
$163A^{16}$–2($X_{Sd}X_{Sd}$)	$163A^{16}$–11($X_{Sd}Y_{Sr}$)	$163A^{17}$b	5/20/60	3	6	0
$163A^{16}$–2($X_{Sd}X_{Sd}$)	$163A^{16}$–11($X_{Sd}Y_{Sr}$)	$163A^{17}$b	6/17/60	10	6	0

* The superscript number refers to the number of the inbred generation. The number following the hypen refers to an individual member of the pedigree.

tirely of females, *viz.* 11 *Sp* and nine *SpSr*. Two other broods produced by the same male with a different female were normal. In a $163A^{14}$ brood, eight exceptional *SdSr* females were produced, in addition to five normal females and two normal males. These were the only broods of strains 163A and 163B raised in that room that fall.

Several normal broods of both strains were subsequently raised. Then, in the spring of 1960, a brood of 163A of the 17th inbred generation was found to contain five exceptional females in addition to four normal females and four normal males (Table 1). Two broods from the same parents born later that spring were normal. In two $163A^{17}$ broods produced by the same male with a different female, one exceptional female and 18 normal females and ten normal males were produced. Forty-two $163B^{16}$ fish raised at the same time in adjacent tanks were normal.

No sex reversals were found among the very closely related fish of strains 163A and 163B raised in an adjoining room during the same period. As far as known, the only difference between conditions in the two rooms is that the latter has glass rather than plastic skylights.

Matings of sex-reversed females

The 163A[14] and 163B[12] exceptional females, all of which were maintained alive to old age, had the external appearance of normal females; the gonads of two that were sectioned were normal ovaries; and all that were exposed to males (about one half) produced young. The 127 offspring of three of the $X_{Sd}Y_{Sr}$ females, mated to normal $X_{Sp}Y_{Sr}$ males, are classified in Table 2. The results

TABLE 2

Offspring of sex-reversed XY females mated to normal XY males

Parents		Pedigree number	Offspring			
Female	Male		$X_{Sp}X_{Sd}$ ♀♀	$X_{Sp}Y_{Sr}$ ♂♂	$X_{Sd}Y_{Sr}$ ♂♂	$X_{Sr}Y_{Sr}$ ♂♂
$163A^{14}$–1($X_{Sd}Y_{Sr}$)*	$163B^{12}$–11($X_{Sp}Y_{Sr}$)	935a	8	16	11	10
$163A^{14}$–2($X_{Sd}Y_{Sr}$)	$163B^{12}$–11($X_{Sp}Y_{Sr}$)	935b	6	10	11	14
$163A^{14}$–3($X_{Sd}Y_{Sr}$)	$163B^{12}$–12($X_{Sp}Y_{Sr}$)	939	8	15	10	8
			22	41	32	32

* The superscript number refers to the number of the inbred generation. The number following the hyphen refers to an individual member of the pedigree.

conform statistically to a 1:1:1:1 ratio of *SpSd* females, *SpSr* males, *SdSr* males, and *SrSr* males. As each sex chromosome involved in these matings was marked by a macromelanophore gene, it can be seen that the XX offspring are females and the XY and YY offspring males. Thus, there is a return to the usual sex-determining mechanism.

Crossing over of macromelanophore genes from one sex chromosome to the other occurs occasionally in *X. maculatus* (GORDON 1951). If the exceptional fish had been the result of crossing over, the *SpSr* offspring would have been females.

The exceptional $163A^{17}$ females also looked and behaved like normal females; offspring from matings of these females are being raised.

Inheritance of shoulder-spot pattern

The shoulder-spot pattern consists of one or two round black areas dorsal to the pectoral fin, formed by a very dense accumulation of micromelanophores in the dermis (Figure 1). It occurs in some individuals in various strains of *X. maculatus*. Its inheritance appears to be complex; only in the inbred strains 163A and 163B has it been found to follow a definite pattern of occurrence. In these strains it normally occurs in all the females but in none of the males. In the crosses involving sex-reversed fish, the shoulder-spot pattern did not appear in XY females. Thus, the appearance of the pattern in these strains appears not to be dependent upon female hormones, but rather upon either the presence of two X chromosomes or the absence of a Y chromosome. Moreover, females treated from birth with methyl testosterone showed no diminution of the shoulder-spot pattern.

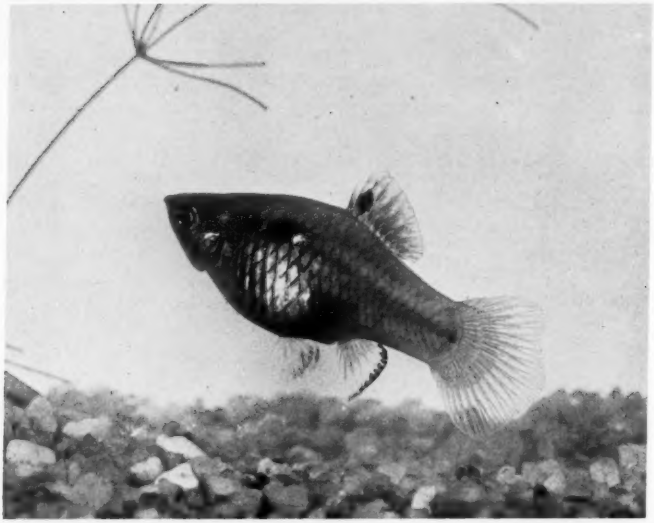


FIGURE 1.—Strain 163A female, showing the *Sp* (spotted-side) macromelanophore pattern and the shoulder-spot micromelanophore pattern (the round, black area dorsal to the pectoral fin).

DISCUSSION

Among the tens of thousands of *X. maculatus*, belonging to many wild and domesticated strains, that have been raised in this laboratory since 1939, only two instances of sex reversal have previously been observed: (1) one wild XX male, whose prematurely sterile testis showed various abnormalities (GORDON 1947; GORDON and ARONOWITZ 1951); and (2) two XX males of the domesticated BH strain (BAKER-COHEN, unpublished data).

BREIDER (1942) described an exceptional WY male in a domesticated platyfish stock. BELLAMY and QUEAL (1951) described the rare occurrence in their laboratory of WY males of domesticated stock. ÖKTAY (1959) found an exceptional XX male among the offspring of a cross of an XX female, which belonged to strain 163, with an XY male, which was a hybrid (strain 163 × a domesticated strain). By inbreeding this male and its offspring, a large number of XX males were produced. ÖKTAY (1959) also described two WY males in the same domesticated strain.

In the medaka, *Oryzias latipes*, AIDA (1936) described several XX males and two XY females; by inbreeeding the former the proportion of XX males was increased. YAMAMOTO (1955, 1959) reported the rare spontaneous occurrence of some XY female medakas. In the guppy, *Lebistes reticulatus*, WINGE (1930, 1934) found a few XX males and by inbreeding eventually produced large numbers of XX males. He also (1934) reported an XY female.

It has generally been postulated that these cases of sex reversal were the result

of genic imbalance, which arose from an accumulation of feminine or masculine genes in the autosomes (WINGE 1934; YAMAMOTO 1959; ÖKTAY 1959), a "fluctuating potency" of the sex chromosomes (AIDA 1936), or a fortuitous combination of genes on the sex chromosomes and autosomes (GORDON and ARONOWITZ 1951). WINGE (1934) and AIDA (1936) also suggested that sex determination may be influenced in the male direction in the summer.

In the present instances, several factors should be noted. The species and strains involved were well established as to their stability in sex determination. A number of sex reversals suddenly appeared, but only in certain broods and usually not in all fish in a brood. Some time later, several additional cases appeared in the same strains; these were not direct descendents of the previous fish. All reversals were in the female direction; the females appeared to be morphologically and functionally normal. The sex of their offspring followed the normal pattern. No sex reversals were found among the closely related fish of these strains raised at the same time in another room.

An explanation of the above may be that in these strains, due to inbreeding, the male-tendency and female-tendency genes have become quite closely balanced, thus making certain individuals susceptible to the influence of exogenous factors. Or this phenomenon may be a reflection of the poorly canalized development and increased environmental variability often found in inbred organisms (LERNER 1954).

What exogenous factors may be involved is yet unknown, but the season of the year is apparently unimportant. Any such factor would have to act on the fish before birth, since, in this species, sex is definitely determined by birth and can not then be transformed, even by the administration of sex hormones (TAVOLGA 1949). The medaka, on the other hand, differentiates sexually after birth, and YAMAMOTO (1953, 1955, 1958, 1959) has successfully raised numbers of males and females that had been sex-reversed by treatment with sex hormones.

SUMMARY

Sex reversals are rare in the platyfish, *Xiphophorus maculatus*. However, 23 sex reversals, which were phenotypic females but genotypic males, appeared spontaneously within a period of one and one half years in two closely related, inbred strains which had shown, since their collection in 1939, a stable XX-female, XY-male sex-determining mechanism. When mated to XY males, the sex-reversed females produced XX females and XY and YY males.

ACKNOWLEDGMENTS

I wish to thank DRS. JAMES W. ATZ and KLAUS D. KALLMAN for reading the manuscript critically and the latter for translating the German references.

LITERATURE CITED

AIDA, T., 1936 Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. Genetics **21**: 136–153.

BELLAMY, A., and M. QUEAL, 1951 Heterosomal inheritance and sex determination in *Platypoecilus maculatus*. Genetics **36**: 93–107.

BREIDER, H., 1942 ZW-Männchen und WW-Weibchen bei *Platypoecilus maculatus*. Biol. Zentr. **62**: 187–195.

GORDON, M., 1947 Genetics of *Platypoecilus maculatus*. IV. The sex determining mechanism in two wild populations of the Mexican platyfish. Genetics **32**: 8–17.

1950 Fishes as laboratory animals. Pp. 345–449. *The Care and Breeding of Laboratory Animals*. Edited by E. FARRIS. John Wiley & Sons. New York.

1951 Genetic and correlated studies of normal and atypical pigment cell growth. Growth Symposia **10**: 153–219.

GORDON, M., and O. ARONOWITZ, 1951 Sex determination in *Platypoecilus maculatus*. II. History of a male platyfish that sired all female broods. Zoologica **36**: 147–153.

KALLMAN, K., 1960 Dosage and additive effects of histocompatibility genes in the teleost *Xiphophorus maculatus*. Ann. N. Y. Acad. Sci. **87**: 10–43.

LERNER, I. M., 1954 *Genetic Homeostasis*. Oliver & Boyd. Edinburgh.

ÖKTAY, M., 1959 Über Ausnahmemaennchen bei *Platypoecilus maculatus* und eine neue Sippe mit XX- Maennchen und XX- Weibchen. Rev. fac. sci. univ. Istanbul, Sér. B. **24**: 75–92.

TAVOLGA, M., 1949 Differential effects of estradiol, estradiol benzoate and pregneninolone on *Platypoecilus maculatus*. Zoologica **34**: 215–237.

WINGE, Ö., 1930 On the occurrence of XX males in Lebistes, with some remarks on Aida's so-called "non-disjunctional" males in Aplocheilus. J. Genet. **23**: 69–76.

1934 The experimental alteration of sex chromosomes into autosomes and vice versa, as illustrated by Lebistes. Compt. rend. trav. lab. Carlsberg. Sér. physiol. **21**: 1–49.

YAMAMOTO, T., 1953 Artificially induced sex-reversal in genotypic males of the medaka (*Oryzias latipes*). J. Exptl. Zool. **123**: 571–594.

1955 Progeny of artificially induced sex-reversals of male genotype (XY) in the medaka (*Oryzias latipes*) with special reference to YY-male. Genetics **40**: 406–419.

1958 Artificial induction of functional sex-reversal in genotypic females of the medaka (*Oryzias latipes*). J. Exptl. Zool. **137**: 227–264.

1959 A further study on induction of functional sex reversal in genotypic males of the medaka (*Oryzias latipes*) and progenies of sex reversals. Genetics **44**: 739–757

GENETICS SOCIETY OF AMERICA

EXECUTIVE COMMITTEE

President, BERWIND P. KAUFMANN — *Vice-President*, RAY D. OWEN

Past Presidents, KARL SAX, JAMES F. CROW

Secretary, W. L. RUSSELL
Oak Ridge National Lab.
Oak Ridge, Tenn.

Treasurer, DREW SCHWARTZ
Oak Ridge National Lab.
Oak Ridge, Tenn.

"The Genetics Society of America is organized to provide facilities for association and conference among students of heredity and for the encouragement of close relationship between workers in genetics and those in related subjects. All persons actively interested in any field of genetics shall be eligible to active membership. Candidates for membership must be recommended by two members of the Society. Members shall be elected by the Executive Committee." —Constitution, Article I.

Any one interested in joining the Society should write to the Secretary.

The Genetics Society publishes annually the Records of the Society containing the program and abstracts of the Annual Meeting and other items of interest to members, including a membership list at periodic intervals. Total membership of the Society was 1217 in May, 1960. Copies of the Records may be obtained from the Treasurer for $1.00. The Genetics Society has a representative on the Editorial Board of GENETICS.

SUSTAINING MEMBERS

The Genetics Society of America established in 1949 a new type of membership, Sustaining Members. The constitution provides that "All organizations interested in any field of genetics shall be eligible to sustaining membership." The dues for sustaining members are $50.00 a year.

Money contributed by Sustaining Members is not used for running expenses of the Society, but is used for special purposes as decided by the Executive Committee. At the meeting of the Executive Committee on August 29, 1960, $500.00 was allocated to GENETICS from the Sustaining Membership Fund.

The following organizations became sustaining members of the Society in the years indicated:

Animal Breeding Consultants
Rt. 1, Box 97B.
Livermore, California—1958

Asgrow Seed Company
272 George Street
New Haven 2, Conn.—1950

Bear Hybrids Corn Co.
Decatur, Ill.—1950

Bristol Laboratories, Inc.
P. O. Box 657
Syracuse 1, New York—1957

W. Atlee Burpee Co.
Philadelphia 32, Pa.—1950

Colonial Poultry Farms, Inc.
Pleasant Hill, Missouri—1958

DeKalb Agric. Assoc., Inc.
DeKalb, Illinois—1949

Ferry-Morse Seed Co.
Detroit 31, Mich.—1949

Funk Brothers Seed Co.
Bloomington, Ill.—1950

Ghostley's Poultry Farm
Anoka, Minnesota—1956

Greenwood Seed Company
Greenwood Farms
Thomasville, Georgia—1959

Kimber Farms, Inc.
Niles, California—1953

Moews Seed Co.
Granville, Ill.—1949

Nicholas Oak Hills Research Farm, Inc.
Sonoma, California—1958

Nichols Incorporated
Marlborough Road
Glastonbury, Connecticut—1950

Northrup King & Co.
Minneapolis 15, Minn.—1957

Pfister Associated Growers, Inc.
Aurora, Ill.—1957

Pioneer Hi-Bred Corn Co.
Johnston, Iowa—1949

This page is donated by GENETICS to the GENETICS SOCIETY OF AMERICA. The society and the journal are separate organizations with a common bond of interest in furthering the science of heredity.

WILEY BOOKS

DEVELOPMENTAL GENETICS AND LETHAL FACTORS

By Ernst Hadorn, *University of Zürich*. Lethal factors occupy a special position in biological research. Since a high proportion of the mutations in all organisms are lethals, they contribute a large body of material which needs to be incorporated into any general theory of the gene and its mutability. While Mendelian lethal factors demonstrate the role played by the genetic material of the chromosomes, each newly arisen lethal mutant sets up a highly specific experiment, showing the functional relationships between individual mutational states and the processes leading to the formation of characters. The explicit purpose of this book, then, is to provide a contribution to the general and special pathology of development, based on an intensive study of lethal factors, and should provide a better understanding of the gene physiological basis of normal development. 1961. 355 pages. $8.50.

MICROBIAL CELL WALLS

By Milton R. J. Salton, *University of Manchester, Great Britain*. This book makes available the 1960 CIBA Lectures in Microbial Biochemistry presented at the Institute of Microbiology, Rutgers University. 1961. 94 pages. $3.50.

JOHN WILEY & SONS, INC.
440 Park Avenue South, New York 16, New York

ECOLOGY
VOL. 42, No. 2 April, 1961

CONTENTS

Dispersal in the red flour beetle *Tribolium castaneum* (Tenebrionidae) ..... Alfred F. Naylor
Turnover-time and production of the planktonic crustacea in limed and reference portion of a bog lake ..... Raymond G. Stross, John C. Neess and A. D. Hasler
Respiratory energy loss and activity patterns in the meadow vole, *Microtus pennsylvanicus pennsylvanicus* ..... Richard G. Wiegert
The relation of energy requirements of tropical finches to distribution and migration ..... George W. Cox
Population dynamics of some freshwater zooplankton ..... Donald H. Hazelwood and Richard A. Parker
Reproductive ecology and sound production of the toadfish, *Opsanus tau* ..... Grace-Ann Gray and Howard E. Winn
The contribution of alder trees (*Alnus tenuifolia*) to the primary productivity of Castle Lake, California ..... Charles R. Goldman
Some dynamics of spatial distribution within semi-natural populations of prairie deermice ..... C. Richard Terman
Insect-vegetaiton relationships in an area contaminated by radioactive wastes ..... D. A. Crossley, Jr., and Henry F. Howden
Growth and reproduction of the oyster drill *Eupleura caudata* in the York River, Virginia ..... Clyde L. MacKenzie, Jr.
The microbiology of Flathead and Rogers Lakes, Montana. II. Vertical distribution of the microbial populations and chemical analyses of their environments ..... Louise F. Potter and Gladys E. Baker
The chaparral formation of Southeastern Oregon, with considerations of its postglacial history ..... Leroy E. Detling
The spruce-fir forests of the Keweenaw Peninsula, Northern Michigan ..... Paul F. Maycock
Flowering and fruiting in the white oaks. I. Staminate flowering through pollen dispersal ..... Ward M. Sharp and Henry H. Chisman
Seasonal trends in carbon dioxide absorption, cold resistance, and transpiration of some evergreens ..... Johnson Parker
Facultative heterotrophy in certain soil algae from the ecological viewpoint ..... Bruce C. Parker
Plant ecology of the teak plantations in Trinidad ..... Philip Ross

ANNALS OF
HUMAN GENETICS
formerly
(ANNALS OF EUGENICS)
EDITED BY
L. S. PENROSE
with the assistance of
JULIA BELL • R. A. FISHER
J. B. S. HALDANE • R. R. RACE
M. N. KARN • J. A. F. ROBERTS
C. A. B. SMITH

Subscriptions in advance
$17.50 per volume of 4 parts

Orders should be placed with
CAMBRIDGE UNIVERSITY PRESS
32 East 57th Street, New York 22

INFORMATION FOR CONTRIBUTORS

(Authors should read New Policies. GENETICS **45**:7–9, 1960 for details)

GENETICS accepts original contributions in genetics proper, or in any scientific field of primary genetic interest.

Ordinarily, manuscripts will appear in print in the order they were received, but *Short* and *Long* papers will be processed on different printing schedules with the short papers appearing at an accelerated rate. ***Short*** papers can be no longer than *four* printed pages; they must conform to the standards and follow the form required for regular papers. Long papers will be limited to ***20*** printed pages. A paper longer than 20 pages will be published only by special vote of the Editorial Board and the author will be expected to bear the costs of the additional pages.

An author must submit to the Editors **two** copies of a manuscript, **typewritten** with **double spacing** throughout. All manuscripts must conform to the general usage in GENETICS particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a "Summary". A symbol (gene, for example) should be defined or identified the first time it is used. Excessive footnotes should be avoided. Footnotes to text statements, if necessary, can usually be included in the text parenthetically. Tables must be typewritten on separate pages with double spacing throughout, arranged to journal page size ($5 \times 7\frac{3}{8}$), and in a form acceptable to the journal and suitable for the printer to set. (See New Policies, GENETICS **45**:7–9, 1960.) Legends for figures and plates are to be typewritten, double spaced, on separate pages. Material for figures should be original drawings (clear photographs usually are satisfactory for line drawings) and should be of a size permitting slight reduction for the printed page. Illustrations should be mounted in final form for engraving. Photographs and drawings substantially larger than a typewritten page are likely to be damaged in the mail and should not be sent. Reviewing of manuscripts is facilitated if additional photographic copies of figures are sent. Chemical structural formulas, complex mating-type charts, and other sketches must be in form that can be photographed. (Clear photographs are acceptable.) Complex mathematical formulas are preferred in the form of reproducible photographs; otherwise they must be typewritten. (See GENETICS **45**:7–9, 1960.)

The manuscript of a published paper will not be returned unless the author so requests.

Galley proof will be sent to authors; page proof will not be sent. Authors should leave forwarding directions, if necessary, or should make other arrangements to have the galley proof corrected promptly. Send corrected proof to the Editors.

Reprints are to be ordered directly from the Editors at the time galley proof is returned. The author will be billed in accordance with a schedule of charges appearing on the reprint order blank. Copies of the schedule are sent to the author along with galley proof.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to be applied toward the cost of reproducing illustrations and of printing expensive tables and formulas.

Manuscripts and correspondence should be addressed to the Editors of GENETICS, Experimental Science Building 122, University of Texas, Austin 12, Texas.

CONTENTS OF PRECEDING ISSUES

FEBRUARY, 1961

WALEN, KIRSTEN H., Studies of cell lethality of a small deficiency in *Drosophila melanogaster*.

KUBITSCHEK, H. E., and H. E. BENDIGKEIT, Latent mutants in chemostats.

CAPPS, ANN SCHLESSELMAN, The effects of nitric oxide on radiation damage in *Drosophila virilis* and *Drosophila melanogaster*.

SPALDING, J. F., V. G. STRANG, and W. L. LESTOURGEON, Heritability of radiation damage in mice.

MAGUIRE, MARJORIE P., Pachytene pairing failure in corn in two inbred lines and their hybrid.

BROWN, E. H., and R. C. KING, Studies on the expression of the transformer gene of *Drosophila melanogaster*.

TOKUNAGA, CHIYOKO, The differentiation of a secondary sex comb under the influence of the gene engrailed in *Drosophila melanogaster*.

STALKER, HARRISON D., The genetic systems modifying meiotic drive in *Drosophila paramelanica*.

ALTENBURG, EDGAR, and LUOLIN S. BROWNING, The relatively high frequency of whole-body mutations compared with fractionals induced by X-rays in Drosophila sperm.

MULLER, H. J., ELOF CARLSON, and ABRAHAM SCHALET, Mutation by alteration of the already existing gene.

MARCH, 1961

TANTAWY, A. O., Effects of temperature on productivity and genetic variance of body size in populations of *Drosophila pseudoobscura*.

DAWOOD, M. M., The genetic load in the second chromosomes of some populations of *Drosophila melanogaster* in Egypt.

VAHARU, TIIU, Modification of ultraviolet-induced mutation frequency in *Neurospora crassa*.

EVANS, H. J., Chromatid aberrations induced by gamma irradiation. I. The structure and frequency of chromatid interchanges in diploid and tetraploid cells of *Vicia faba*.

EVANS, H. J., and T. R. L. BIGGER, Chromatid aberrations induced by gamma irradiation. II. Non randomness in the distribution of chromatid aberrations in relation to chromosome length in *Vicia faba* root-tip cells.

MOUTSCHEN, J., Differential sensitivity of mouse spermatogenesis to alkylating agents.

STEINER, ERICH, Incompatibility in the complex-heterozygotes of Oenothera.

PHILLIPS, J. N., JR., Modification of mutation frequency in microorganisms. I. Preirradiation and postirradiation treatment.

BRYAN, C. R., and M. R. IRWIN, The relationships of cellular antigen C of *Columba guinea* to antigenic characters in other species of Columbidae.

BROSSEAU, G. E., B. NICOLETTI, E. H. GRELL, and D. L. LINDSLEY, Production of altered Y chromosomes bearing specific sections of the X chromosome in Drosophila.

LOOSLI, ROLF, ELIZABETH S. RUSSELL, WILLYS K. SILVERS, and JANICE L. SOUTHARD, Variability of incidence and clinical manifestation of mouse hereditary muscular dystrophy on heterogeneous genetic backgrounds.

APRIL, 1961

WHITTINGHILL, MAURICE, and DAVID GALE DAVIS, Increased recombination from female Drosophila irradiated as larvae without oocytes.

FAHMY, O. G., and MYRTLE J. FAHMY, Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*. IX. The cell-stage response of the male germ line to the mesyloxy esters.

FROST, JUSTIN N., Preferential segregations in triploid females.

APIRION, DAVID, and DANIEL ZOHARY, Chlorophyll lethal in natural populations of the orchard grass (*Dactylis glomerata* L.). A case of balanced polymorphism in plants.

BRNCIC, DANKO, Non random association of inversions in *Drosophila pavani*.

BROWN, WILLIAM P., and A. E. BELL, Genetic analysis of a "plateaued" population of *Drosophila melanogaster*.

WADDLE, B. M., C. F. LEWIS, and T. R. RICHMOND, The genetics of flowering response in cotton. III. Fruiting behavior of *Gossypium hirsutum* race *latifolium* in a cross with a variety of cultivated American Upland cotton.

WHITING, P. W., *R*-locus eye-color factors in Mormoniella.

FAHMY, O. G., and MYRTLE J. FAHMY, Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*. X. The nature of the mutations induced by the mesyloxy esters in relation to molecular cross-linkage.

SINGLE NUMBERS $1.50 ANNUAL SUBSCRIPTION $12.00

obtainable from

GENETICS, INC., BUSINESS OFFICE, EXPERIMENTAL SCIENCE BLDG. 122,
UNIVERSITY OF TEXAS, AUSTIN 12, TEXAS